Electrophysiological Markers for Neuropathic Pain in Spinal Cord Injured Subjects

Abstract

Physical disability following spinal cord injury (SCI) is the most striking problem noted by the general public. But for the affected subjects urogenital difficulties or depression and pain are often more burdensome. Pain after SCI can have various reasons but only neuropathic pain below the level of lesion (bNP) is thought to be caused by injury of the spinal nervous tissue. This type of pain is in the focus of this thesis. Once bNP has established it is mostly chronic and medication is generally ineffective. Currently, more and more treatments trying to restore function after SCI enter the clinical trial phase. Besides improving function, however, treatments increasing nerve growth in the spinal cord risk to induce or exacerbate bNP. Therefore, observation of bNP is a crucial factor in such interventional studies. A method to objectively supervise bNP has, however, not yet been established. The spinothalamic tract (STT) mainly transmits nociceptive and temperature information in the spinal cord. This tract was dysfunctional in SCI subjects suffering from bNP in clinical examinations. Nevertheless, STT dysfunction was not predictive for bNP and sensory differences between subjects with and without bNP could not be detected. In contrast to clinical examination which is always subjective and only offers limited resolution, electrophysiological measures allow for a more detailed and objective investigation. The novel electrophysiological method of contact heat evoked potentials (CHEP) measures STT function. Establishment of this method was the goal of the first study. The painful stimulation on locations along the spine allowed the calculation of the conduction velocity of the STT in healthy subjects. Furthermore the CHEP latency depended linearly on the heat pain threshold with 1° C higher threshold leading to approximately 10 ms longer latency. It was hypothesized that the rather low heating rate combined with the time-consuming passive heat spread from skin surface to nociceptors was responsible for this. The second study aimed at clarifying this dependence through comparison of the results of study 1 with those of a theoretical heat transfer model. According to this model, 1° C higher pain threshold leads to approximately 15 ms longer CHEP latency. The close similarity between the experimentally determined (study 1) and the computed dependence, proved the influence of the pain threshold on CHEP latency. Summary Electrophysiological markers for Neuropathic Pain in SCI Subjects 2 Subjects suffering from neuropathic pain (NP) in general and not only in SCI, have lowered EEG peak frequency. It was hypothesized in literature that the reduced EEG peak frequency emerged from thalamic deafferentiation and from the ensuing dysrhythmia in thalamocortical feedback loops. Therefore, the third study investigated EEG peak frequency in addition to STT function and compared both between SCI subjects with and without bNP and controls. The STT function (measured with CHEP) below the level of injury was distinctly impaired in SCI compared to control subjects. Furthermore, the EEG peak frequency was generally lower in the SCI subjects. While the CHEP measurements did not reveal differences between subjects with and without bNP, the EEG peak frequency was lowered in subjects with bNP. This difference, however, was only apparent after the linear dependence of EEG peak frequency from the level of SCI was taken into account. In consideration of this dependence, the EEG peak frequency could in future be helpful to supervise bNP both in studies aiming at restoring function or reducing pain after SCI. Currently, the clinical read-out parameter for STT function is pinprick sensation. In the fourth study this pinprick sensation was traced over the first year after SCI. Comparison of this STT function with the bNP state of the same subjects 2-5 years after SCI disclosed larger functional STT recovery in subjects suffering from bNP. Despite the different STT functional recovery, the initial and end measurements did not discriminate between subjects with and without bNP. This was in agreement with earlier studies. The results corroborate the above mentioned hypothesis that new therapies intending to promote sensorimotor recovery after SCI could simultaneously induce bNP by boosting recovery of spinothalamic function.

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Presented by
SUSANNE WYDENKELLER
Dipl. Natw. ETH

Date of birth
7 July 1981

Citizen of
Winterthur (ZH)

Accepted on the recommendation of
Prof. Dr. Martin E. Schwab
Prof. Dr. Robert Riener
Dr. Pascal Halder
Dr. Julia Blum

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Table of Contents

Summary

Physical disability following spinal cord injury (SCI) is the most striking problem noted by the general public. But for the affected subjects urogenital difficulties or depression and pain are often more burdensome. Pain after SCI can have various reasons but only neuropathic pain below the level of lesion (bNP) is thought to be caused by injury of the spinal nervous tissue. This type of pain is in the focus of this thesis. Once bNP has established it is mostly chronic and medication is generally ineffective. Currently, more and more treatments trying to restore function after SCI enter the clinical trial phase. Besides improving function, however, treatments increasing nerve growth in the spinal cord risk to induce or exacerbate bNP. Therefore, observation of bNP is a crucial factor in such interventional studies. A method to objectively supervise bNP has, however, not yet been established.

The spinothalamic tract (STT) mainly transmits nociceptive and temperature information in the spinal cord. This tract was dysfunctional in SCI subjects suffering from bNP in clinical examinations. Nevertheless, STT dysfunction was not predictive for bNP and sensory differences between subjects with and without bNP could not be detected. In contrast to clinical examination which is always subjective and only offers limited resolution, electrophysiological measures allow for a more detailed and objective investigation.

The novel electrophysiological method of contact heat evoked potentials (CHEP) measures STT function. Establishment of this method was the goal of the first study. The painful stimulation on locations along the spine allowed the calculation of the conduction velocity of the STT in healthy subjects. Furthermore the CHEP latency depended linearly on the heat pain threshold with 1° C higher threshold leading to approximately 10 ms longer latency. It was hypothesized that the rather low heating rate combined with the time-consuming passive heat spread from skin surface to nociceptors was responsible for this.

The second study aimed at clarifying this dependence through comparison of the results of study 1 with those of a theoretical heat transfer model. According to this model, 1° C higher pain threshold leads to approximately 15 ms longer CHEP latency. The close similarity between the experimentally determined (study 1) and the computed dependence, proved the influence of the pain threshold on CHEP latency.

Subjects suffering from neuropathic pain (NP) in general and not only in SCI, have lowered EEG peak frequency. It was hypothesized in literature that the reduced EEG peak frequency emerged from thalamic deafferentiation and from the ensuing dysrhythmia in thalamocortical feedback loops. Therefore, the third study investigated EEG peak frequency in addition to STT function and compared both between SCI subjects with and without bNP and controls. The STT function (measured with CHEP) below the level of injury was distinctly impaired in SCI compared to control subjects. Furthermore, the EEG peak frequency was generally lower in the SCI subjects. While the CHEP measurements did not reveal differences between subjects with and without bNP, the EEG peak frequency was lowered in subjects with bNP. This difference, however, was only apparent after the linear dependence of EEG peak frequency from the level of SCI was taken into account. In consideration of this dependence, the EEG peak frequency could in future be helpful to supervise bNP both in studies aiming at restoring function or reducing pain after SCI.

Currently, the clinical read-out parameter for STT function is pinprick sensation. In the fourth study this pinprick sensation was traced over the first year after SCI. Comparison of this STT function with the bNP state of the same subjects 2-5 years after SCI disclosed larger functional STT recovery in subjects suffering from bNP. Despite the different STT functional recovery, the initial and end measurements did not discriminate between subjects with and without bNP. This was in agreement with earlier studies. The results corroborate the above mentioned hypothesis that new therapies intending to promote sensorimotor recovery after SCI could simultaneously induce bNP by boosting recovery of spinothalamic function.

Zusammenfassung

In der Wahrnehmung der breiten Öffentlichkeit ist die Gehbehinderung nach einer Rückenmarkverletzung das augenfälligste Problem. Für die Betroffenen hingegen sind Blasen- und Kontinenzprobleme, sowie Schmerz und Depression oft belastender. Schmerz in Rückenmarkverletzten kann verschiedene Gründe haben, doch nur neuropathischer Schmerz unterhalb der Verletzungshöhe (bNP) wird auf die Rückenmarksverletzung zurückgeführt. Diese Schmerzart steht im Zentrum der vorliegenden Dissertation. Einmal etabliert, ist bNP meist chronisch und Medikamente sind oft nicht genügend wirksam. Im Moment kommen mehr und mehr Behandlungen mit dem Ziel Funktionsverbesserungen nach der Verletzung zu erzielen in die klinische Testphase. Neben einer Funktionsverbesserung könnten solche, das Nervenwachstum im Rückenmark fördernde, Behandlungen aber auch bNP verursachen/verstärken. Bis jetzt ist eine objektive bNP Überwachung nicht möglich, könnte aber in klinischen Studien eine wichtige Rolle spielen.

Klinische Untersuchungen haben ergeben, dass der spinothalamische Trakt (STT), der hauptsächlich nozizeptive und Temperatur Information im Rückenmark überträgt, in Rückenmarksverletzten mit bNP nicht normal funktioniert. Trotzdem war ein STT Schaden nicht vorhersagend für bNP und es konnten keine sensorischen Unterschiede zwischen Rückenmarkverletzten mit und ohne bNP gefunden werden. Im Gegensatz zur klinischen Untersuchung, die immer subjektiv ist und nur eine beschränkte Auflösung bietet, könnten elektrophysiologische Messungen eine detailliertere und objektivere Untersuchung erlauben.

Ziel der ersten Studie war es, die neuartige elektrophysiologische Methode zur Messung von Kontakthitze evozierten Potentialen (CHEP) für die STT Funktionsanalyse zu etablieren. Die schmerzhafte Stimulation an verschiedenen Punkten auf dem Rücken erlaubte die Berechnung der STT Leitgeschwindigkeit in gesunden Probanden. Ausserdem hing die CHEP Latenz linear von der Hitzeschmerzschwelle ab. Eine um 1° C höhere Schwelle führte zu 10 ms verlängerter Latenz. Die eher tiefe Heizrate zusammen mit der zeitintensiven passiven Hitzeleitung von der Hautoberfläche zu den Nozizeptoren könnte für diesen Effekt verantwortlich sein.

Die zweite Studie hatte zum Ziel diese Abhängigkeit durch einen Vergleich mit einem theoretischen Wärmeleitmodell zu verdeutlichen. Gemäss diesem Modell führt eine um 1° C höhere Schmerzschwelle zu ca. 15 ms längerer Latenz. Die Ähnlichkeit zwischen der berechneten und gemessenen (Studie 1) Abhängigkeit bestätigte den Einfluss der Schmerzschwelle auf die CHEP Latenz.

Bei Patienten mit neuropathischem Schmerz im Allgemeinen ist eine verringerte EEG Peakfrequenz bekannt. In der Literatur wurde spekuliert, dass diese reduzierte Frequenz von der Deafferenzierung des Thalamus und der resultierenden Dysrhythmie in thalamokortikalen Verbindungen herrühren könnte. Darum untersuchte die dritte Studie die EEG Peakfrequenz zusätzlich zur Funktion des STT (mit CHEP) und verglich beide zwischen einer Kontrollgruppe und Rückenmark-verletzten mit und ohne bNP. Verglichen mit Kontrollpersonen hatten die Quer-schnittverletzten eine deutlich beeinträchtige STT Funktion unterhalb der Verletzung. Ausserdem war die EEG Peakfrequenz in den Rückenmarkverletzten tiefer als in der Kontrollgruppe. Während die CHEP Messungen keine Unterschiede zwischen Rückenmarkverletzten mit und ohne bNP zeigten, war die EEG Peakfrequenz in den Patienten mit bNP deutlich verringert. Dieser Unterschied wurde aber erst signifikant nachdem die lineare Abhängigkeit der EEG Peakfrequenz von der Verletzungshöhe berücksichtigt und kontrolliert wurde. Wird die Deafferenzierung berücksichtigt, könnte zukünftig die EEG Peakfrequenz bei der Überwachung von bNP helfen. Dies sowohl bei Studien, welche versuchen die Funktion zu verbessern, als auch Schmerzen zu verringern.

In der Klinik wird die STT Funktion mit Nadelstich Empfindung beurteilt. Die Entwicklung der STT Funktion wurde in der vierten Studie über das erste Jahr nach Rückenmarksverletzung verfolgt. Ein Vergleich zwischen der STT Funktionsver-besserung mit dem bNP Status 2-5 Jahre nach der Rückenmarksverletzung zeigte eine grössere funktionelle STT Erholung in Personen mit bNP. Im Einklang mit früheren Studien waren die Anfangs- und Endwerte trotz dieser verschiedener funktionellen Erholung in Personen mit und ohne bNP nicht unterschiedlich. Die Resultate könnten jedoch darauf hinweisen, dass neue Behandlungen, welche eine sensomotorische Verbesserung anstreben, gleichzeitig durch eine Erhöhung der STT Erholung auch bNP verursachen könnten.

1 General Introduction

1.1 Spinal Cord Injury

The lesion of the spinal cord through traumatic accidents or non-traumatic causes like tumors or infections can severely impair the life of the affected subject. Depending on the strength and location of the spinal lesion, the symptoms can, however, vary greatly. Apart from the paralysis and sensory impairments, spinal cord injury (SCI) is often associated with pain, urogenital problems and social or economic difficulties. In Switzerland the incidence of SCI caused by trauma or disease lies between 300-400 cases per year (Eberhard, 2004). This results in approximately six new SCI subjects in 100'000 inhabitants a year, what roughly corresponds to values given for other developed countries (Ackery et al., 2004; Hirtz et al., 2007).

While in the past the potential for recovery after SCI was very limited, perspectives look different nowadays (Rossignol et al., 2007). Interventional strategies to improve outcome after SCI include application of drugs or cells to lower the barriers for regeneration and sprouting of axons (Fawcett, 2006; Schwab, 2004; Schwartz and Yoles, 2006). Some of these treatments, however, have the potential to not only allow for the intended functional recovery but also could 'unwanted' fibers sprout aberrantly and lead to complications such as increased pain or spasticity (Deumens et al., 2008; Hofstetter et al., 2005).

1.1.1 Classification of Spinal Cord Injury

Spinal cord injuries are generally classified in terms of the level of lesion and the completeness of the injury. The assessment of spinal cord function is standardized and routinely performed according to the "International Standards for Neurological and Functional Classification of Spinal Cord Injury" established by the American Spinal Injury Association (Marino et al., 2003). The sensory function in all dermatomes is tested as light touch and pinprick sensation at defined key points on both body sides. The sensitivity is scored as 0 (absent), 1 (impaired) or 2 (normal). The motor function of different segments is tested in 10 key muscles and graded from 0-5 (from total paralysis to active movement against full resistance). The most caudal segment of the spinal cord with normal motor and sensory function is then

defined as the neurological lesion level. The severity or completeness of injury is graded into five steps, see Tab. 1.1.

A	Complete	No sensory or motor function is preserved in the sacral segments S4-S5
B	Incomplete	Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-S5.
C	Incomplete	Motor function is preserved below the neurological level, and more than half of the key muscles below the neurological level have a muscle grade less than 3.
D	Incomplete	Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade greater than or equal to 3.
E	Normal	Sensory and motor function is normal.

Tab. 1.1: ASIA impairment scale used for grading the degree of impairment after SCI (Marino et al., 2003)

1.1.2 Measurement of Remaining Spinal Function

Spinal cord function is, in addition to the clinical assessment, measured with well established electrophysiological methods to allow for the diagnosis of neurological deficits. Somatosensory evoked potentials are used to assess function of the ascending dorsal columns and motor evoked potentials to judge function of the descending corticospinal tract. In addition, descending autonomic fibers can be investigated with the somatosensory skin response, for a review, see (Curt and Dietz, 1999) Nevertheless, one main ascending spinal pathway, the spinothalamic tract (STT) is not yet investigated electrophysiologically in clinical routine. The STT conveys nociceptive and temperature information. Its measurement could, besides allowing for a more detailed anatomical description of the spinal injury, help in the assessment of neuropathic pain states (see below).

1.2 Neuropathic Pain

According to the International Association for the Study of Pain, neuropathic pain (NP) is defined as “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system” (Treede et al., 2008). Therefore, NP is not elicited by nociceptive input. Apart from SCI, NP can occur in a wide variety of

disorders involving the nervous system such as multiple sclerosis, stroke, peripheral neuropathy or herpes zoster.

1.2.1 Neuropathic Pain in Spinal Cord Injury

In SCI pain presents a severe burden to the affected subjects over and above their physical disability. Besides NP SCI subjects experience other pain types which are not always easy to differentiate, see Tab. 1.2 (Siddall and Loeser, 2001; Siddall et al., 1997).

The prevalence of NP is high, with roughly 50% of SCI subjects suffering from NP (Siddall et al., 2003; Stormer et al., 1997; Werhagen et al., 2004). The quality of life of the affected subjects is significantly reduced (Anke et al., 1995; Jensen et al., 2005) and the pain is often associated with depression (Cairns et al., 1996). Whereas NP is a common phenomenon, it is not at all described consistently throughout the affected subjects (Putzke et al., 2002). Generally, NP in SCI is classified according to the level of the SCI into above, at and below level NP (Siddall et al., 2000).

	Term	Distinguishing features
Nociceptive	Musculoskeletal	Dull, aching, movement-related, eased by rest, responsive to opioids and NSAIDs, located in musculoskeletal structures
	Visceral	Dull, cramping, located in abdominal region with preserved innervation, also includes dysreflexic headache (vascular)
Neuropathic		Sharp, shooting, burning, electric abnormal responsiveness
	Above level	Located in the region of sensory preservation
	At level	Located in segmental pattern at the level of injury
	Below level	Located diffusely below the level of injury

Tab. 1.2: Grouping of pain types related to SCI (Siddall and Loeser, 2001; Siddall et al., 1997)

While above level NP is not directly related to the SCI and at level NP can also occur due to peripheral nerve damage, below level NP (bNP) is thought to be initiated by the spinal cord lesion, see Tab. 1.2. (Siddall et al., 1997). Since the exact mechanisms of NP generation are not clear, effective pharmacotherapy is difficult and no standard therapy can be applied (Cardenas and Jensen, 2006; Siddall and Loeser, 2001). Therefore, the pain-relief through the applied treatments is mostly insufficient (Widerstrom-Noga and Turk, 2003).

In bNP an involvement of dysfunction of the nociceptive and thermal information transmitting STT is assumed. While STT dysfunction is not predictive for bNP, it seems to be a precondition (Defrin et al., 2001; Eide et al., 1996; Finnerup et al., 2003a; Finnerup et al., 2003b; Finnerup et al., 2007).

1.2.2 Neuropathic Pain and Thalamocortical Dysrhythmia

Another line of NP research focuses on supraspinal mechanisms. Thus, subjects suffering from NP of variable origin exhibited slowed resting brain oscillatory activity (Sarnthein et al., 2006). This brain oscillatory activity is thought to be generated in thalamocortical feedback loops (Llinas et al., 1999; Llinas and Steriade, 2006) and can be quantified by electroencephalogram (EEG) frequency spectra. For EEG, electrodes attached to the scalp measure electrical activity generated by the synchronous activity of large groups of neurons. Frequency spectra of brain oscillatory activity are calculated with fast fourier transformation. The absence of usual input to the thalamic cells involved in the thalamocortical circuits (e.g. through interruption of the spinal afferents) destabilizes these feedback loops. The ensuing thalamocortical dysrhythmia is measurable as slowed EEG peak frequency and thought to be involved not only in NP but also in such diverse conditions as Parkinson's disease, tinnitus or some neuropsychiatric disorders (Llinas et al., 2001). The positive symptoms appearing in these diseases are attributed to high frequency overactivity in cortical structures ultimately resulting from the dysrhythmia (Magnin et al., 2005). Interruption of this vicious circle is hypothesized to reduce the symptoms. Applied to NP, lesion of neurons in medial thalamic structures successfully decreased NP (Jeanmonod et al., 2001; Sarnthein et al., 2006).

In SCI subjects the EEG peak frequency was found to be reduced compared to healthy controls (Boord et al., 2008; Herbert et al., 2007; Tran et al., 2004). However, the EEG peak frequency reduction could not be specifically linked to the occurrence of NP.

1.2.3 Experimental Studies on Neuropathic Pain

Apart from STT dysfunction and thalamocortical dysrhythmia various other mechanisms have been implicated in the generation of NP after SCI. While bNP is caused by central nervous system (CNS) lesion it may share mechanisms with the better investigated NP after peripheral nerve lesion. This holds even more for at level

NP in which a considerable contribution of peripheral mechanisms is assumed. Generally, anatomical, inflammatory, neurochemical and excitotoxic processes, initiated immediately after the injury, were hypothesized to alter the functional state of sensory neurons to produce evoked and spontaneous pain. Mechanisms implicated in the generation of bNP might be rather slow as this NP type only develops as late as on average 1.8 years after the SCI (Siddall et al., 2003). Therefore more time consuming mechanisms such as nerve fiber sprouting or Wallerian degeneration in the CNS, for a review see (Vargas and Barres, 2007), might be involved.

Central sensitization

Sensitization of neurons engaged in pain transmission in the CNS could explain neural hyperexcitability and spontaneous activity typical of NP. Enhanced excitability of central neurons was described after peripheral nerve lesion (Woolf, 1983). But it is also thought to be implicated in central NP after SCI. Thus, the hyperexcitability of wide dynamic range dorsal horn neurons rostral to a complete spinal transsection was paralleled by NP behavior in rats (Zhang et al., 2005). Increased sodium channel expression leading to such hyperexcitable neurons was shown not only in the spinal cord (Hains et al., 2003) but also in the thalamus (Hains et al., 2005) after spinal transsection. Additionally, the neuroimmune cells microglia were found to contribute to SCI NP. After contusive SCI, activated microglia in the spinal cord (also distal to the lesion) can influence neurons over inflammatory mediators (Detloff et al., 2008), for a review see (Ji and Suter, 2007). In this way they are thought to maintain the pain even long after the initial injury (Hains and Waxman, 2006; Zhao et al., 2007a).

The concept of central sensitization is thought to be related to long-term potentiation, a mechanism probably implicated in learning and memory. In long-term potentiation excitatory amino acid receptors exhibit enhanced responsiveness due to phosphorylation. Although most of the insights on excitatory amino acid transmission were gained in peripheral NP, dependence of NP on NMDA (Eide et al., 1995) and non-NMDA receptor (Yezierski et al., 1998; Yezierski et al., 1993) activation was shown also in SCI.

An additional factor contributing to central sensitization might be loss of inhibitory tone due to degeneration of inhibitory interneurons, e.g. in peripheral nerve injuries (Sugimoto et al., 1990). Thus, analgesia in inflammatory pain or peripheral NP can

be achieved by facilitation of GABAergic neurotransmission (Knabl et al., 2008). Recently, enhancement of inhibitory neural activity was also successful against mechanical allodynia in spinally hemi-transected rats through topical application of GABA-A or GABA-B receptor agonists to the spinal cord surface below the lesion level. This intervention also decreased the hyperexcitability of wide dynamic range neurons in the spinal dorsal horn (Gwak et al., 2006). Another explanation for decreased inhibitory tone after SCI can be a lesion of descending serotoninergic projections from the raphe nucleus to neurons in laminae I/II of the dorsal horn. Accordingly, replacement of serotonin below the lesion reduces SCI NP behavior (Hains et al., 2002; Hains et al., 2001).

Sprouting of nerve terminals

After sciatic nerve injury, sprouting of Aβ-fiber collaterals into lamina II has been proposed as a mechanism for hypersensitivity to tactile stimuli (Woolf et al., 1992). Conversely, in SCI NP, another fiber type is mainly thought to sprout in a maladaptive manner. Thus, primary afferents containing calcitonin gene-related peptide (CGRP), that normally synapse on lamina I/II of the dorsal horn, invade laminae III/IV after SCI. This process was associated with SCI NP behavior (Ackery et al., 2007; Christensen and Hulsebosch, 1997). The CGPR containing cells are mostly C-fiber neurons implicated in the transmission of nociceptive and temperature sensation. Accordingly, injection of CGRP receptor antagonist was effective against mechanical and thermal hypersensitivity in rats with spinal hemi-section (Bennett et al., 2000). The presumed mechanism of NP generation would be amplification of the C-fiber input through the increased number of central projections of these fibers.

The findings mentioned here can give valuable information about SCI NP mechanisms. However, they need to be interpreted with caution as some were either determined for peripheral NP only or they were investigated in animal models of SCI. Although standardized measurements for evoked pain such as hyperalgesia and allodynia in experimental animals are applied, controversy about their validity for investigation of bNP exists (Vierck and Light, 2002; Yezierski, 2005). This is because most of the used assessments rather investigate nociceptive reflexes and innate responses than behavioral responses that involve cerebral processing. Furthermore as the experimental animals can not verbally communicate their spontaneous or

evoked sensations, uncertainty about the actual perception of the animal always remains.

1.3 Spinothalamic Tract

Besides its role in the conduction of thermal information, the STT is mainly responsible for conveying nociceptive signals from the periphery to the brain. The nociceptive signals are transmitted in slowly conducting nerve fibers, either the thinly myelinated Aδ- or the unmyelinated C-fibers. The comparably faster conducting Aδ-fibers (10-20 m/s) mediate signals important in the perception of the sharp, stinging first pain while C-fibers (0.1-1 m/s) are involved in the diffuse and longer lasting second pain (Cruccu et al., 2000; Iannetti et al., 2003; Kakigi et al., 1991; Rossi et al., 2000).

1.3.1 Measurement of Spinothalamic Tract Function

Assessment of STT function in current clinical practice is mainly conducted with a pin and judged on a three-point scale (see above). This measurement is subjective and necessitates active participation of the examined subject. Conversely, electrophysiological investigations are more objective and allow a more detailed evaluation.

STT function can be assessed with painful evoked potentials calculated from continuous recording of brain activity (EEG). In these measurements a large amount of background noise due to ongoing brain activity is included. After time-locked averaging according to presentation of a certain stimulus, the background noise is reduced and the stimulus evoked potential appears. The nature of the stimulus is of high importance as only short and clearly defined stimuli can elicit activity synchronous enough for reliable evoked potentials. For excitation of Aδ- and C-fibers of the STT painful skin stimulation is used. Classically, CO_2 laser stimulation was applied, for a review see (Kakigi et al., 2005; Treede et al., 2003). Nowadays a more natural stimulation by contact heat applied through a relatively fast heating thermode is possible, although less established (Arendt-Nielsen and Chen, 2003; Chen et al., 2001). The resulting contact heat evoked potentials (CHEP) have, however, already been proven useful in investigation of diseases such as small fiber neuropathy (Atherton et al., 2007; Chao et al., 2008; Truini et al., 2007).

1.4 Aim of the Thesis

As reviewed in the previous sections, knowledge on bNP in SCI is rather scarce. An involvement of the STT is strongly suspected, nevertheless has differentiation between subjects with and without bNP not yet been achieved. The rather novel electrophysiological method of CHEP might render the assessment of STT function more precise and discriminating. Furthermore, EEG peak frequency was shown to be influenced by SCI and by NP in general, whereas the probably additive influence of the two in SCI subjects has not been elucidated so far. It was thus the aim of this study to:

- Establish the method of contact heat evoked potentials (CHEP) for measurement of STT function in healthy controls and SCI subjects (chapter 2).
- Further elucidate mechanisms influencing CHEP in healthy subjects in order to determine ideal experimental conditions and read-out parameters for everyday clinical practice (chapter 3).
- Show that CHEP allow to measure the STT dysfunction in SCI (chapter 4).
- Determine read-out parameters for bNP in chronic SCI both in terms of CHEP and of EEG peak frequency (chapter 4).
- Investigate the interdependence of STT functional recovery after acute SCI and bNP (chapter 5).

2 Study 1: Contact heat evoked potentials in a normative group[1]

2.1 Abstract

Objective

Laser evoked potentials have been shown to be clinically useful for the electrophysiological assessment of nociceptive pathways. Contact heat evoked potentials (CHEP) are less established but might be advantageous for clinical purposes. This study aimed at determining the conduction velocity (CV) of central pain (spinothalamic tract, STT) pathways using contact heat stimulation in order to replicate previous findings using laser stimulation.

Methods

Contact heat stimulation 3° C higher than the pain threshold was applied at different body locations in 20 subjects.

Results

The CHEP latencies correlated significantly with the respective pain thresholds. Without normalization for this effect no significant linear regression between distance to the brain and the latencies was found. Conversely, if thresholds were considered, the regression was significant and the CV of the STT (ranging between 11.2-13.4 m/s) was comparable to CVs estimated after laser stimulation.

Conclusions

Pain thresholds seem crucial in interpreting CHEP latencies. It is suggested that the rather low heating rate is responsible for the dependence of latencies on the pain thresholds.

[1] This manuscript was published in the journal Clinical Neurophysiology: Spinothalamic tract conduction velocity estimated using contact heat evoked potentials: what needs to be considered; Clinical Neurophysiology 119 (2008) 812–821. The authors were Susanne Wydenkeller, Regula Wirz and Pascal Halder. Most measurements and all analyses were conducted by Susanne Wydenkeller. Regula Wirz contributed the measurements of CHEP to stimulation at threshold +2° C and +4° C. The manuscript was written by Susanne Wydenkeller and revised by the co-authors.

Significance

This study shows the importance of pain thresholds and their control to attain valid CV of the STT after contact heat stimulation in healthy subjects.

2.2 Introduction

The in vivo neurophysiological assessment of the nociceptive pathways in humans by means of laser evoked potentials is an established method for evaluating the integrity of peripheral and central nociceptive pathways (Treede et al., 2003). Brief peripheral application of painful heat stimuli elicits cortical evoked potentials that are transmitted through small Aδ-fibers. Using electroencephalography these potentials can be recorded, in which reduced amplitudes and/or decreased conduction velocity (CV) indicate damage to the small fibers involved. Therefore this technique is clinically useful in the diagnosis of a variety of diseases affecting small fibers (Bromm and Treede, 1991; Iannetti et al., 2001; Lefaucheur et al., 2002; Lefaucheur and Creange, 2004; Spiegel et al., 2003; Treede et al., 1991; Truini et al., 2004). In spinal cord injury (SCI) the detection of damaged fibers in the spinothalamic tract (STT) has further clinical application by permitting a more detailed description of the spinal damage due to the anatomical separation of the STT from the major motor and sensory pathways. Moreover, detection of damage to the STT might be important in the diagnosis of central neuropathic pain (Finnerup et al., 2003b; Garcia-Larrea et al., 2002).

Only recently contact heat stimulators capable of evoking cerebral potentials (Chen et al., 2001) have been developed and are now increasingly used as an alternative to laser stimulation (Granovsky et al., 2005; Iannetti et al., 2006; Valeriani et al., 2002). Although there seems to be general agreement that both methods are suited to activate the nociceptive pathways, some important differences need to be considered when comparing results assessed with the two methods. Compared to laser stimulation, contact heat can be applied with less safety precautions (e.g. no need of an approved room, no safety goggles), the baseline skin temperature can at least be partially controlled and the risk of skin damage is negligible (Arendt-Nielsen and Chen, 2003), all of which could be advantageous for clinical applications. However, normative values for the CV of peripheral and spinothalamic Aδ-fibers after contact heat stimulation have not been reported so far and therefore the basis for clinical applications is not yet established. Although the use of a natural stimulus, such as

contact heat, could offer important insights into human pain perception and physiology, its slow rise time compared to laser stimuli is a clear limitation for studies using event-related potentials. Due to the lower heating rate and the less direct nociceptor activation of contact heat, the latencies evoked by contact heat are usually longer (Baumgartner et al., 2005). More importantly, as pain thresholds can differ within and between subjects, the rather slow heating ramp of contact heat might induce additional latency differences. Those would reflect differences in nociceptor activation time rather than altered CV properties. This aspect has not been investigated so far but could be important to correctly estimate the STT CV.

This study aimed at obtaining normative data on the CV of spinothalamic Aδ-fibers after contact heat stimulation, thereby establishing the preconditions for future clinical applications. Furthermore, this study should clarify whether differences in pain thresholds need to be considered when latencies of contact heat evoked potentials (CHEP) and resulting CVs are interpreted. Finally, cortical sources of the most prominent P2 component were estimated in order to show that contact heat activates cortical areas known to be involved in pain processing.

2.3 Methods

2.3.1 Subjects

Twenty healthy subjects (age 33.0 ± 13.7 years; height 1.79 ± 0.1 m; weight 74.0 ± 12.8 kg; mean ± standard deviation (SD)) participated in the experiment. None of the subjects suffered from acute or chronic pain. One subject was taking anti-epileptic medication but both resting EEG as well as subsequent results did not show any conspicuity and therefore the subject was included in the study. The experiment was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent.

2.3.2 Contact heat stimulation

Pulsed peripheral heat stimuli were applied using a contact heat stimulator (Medoc, Ramat Yishai, Israel) with a constant nominal heating rate (70° C/s) achieved through a heating thermo foil. A peltier element together with an active water cooling system ensured fast cooling down (40° C/s) after arrival at the stimulation temperature. The stimulating area of the thermode was 573 mm^2. Two thermocouples on the thermode

surface measured the temperature at the skin-thermode interface and provided an estimate of the skin temperature. The baseline skin temperature was kept at 35° C. Potential burning of the skin was prevented by the maximally applicable temperature of 55° C. Active heating was stopped 1° C before the intended nominal temperature was reached.

The thermode was applied sequentially to four locations on the left body side: three on the back along the spine (back 1 = upmost; back 2 = middle; back 3 = bottom) lying approximately 5 cm away from the midline and one at the ankle. Through the direct approach using stimulation locations alongside the backbone and thus close and in constant distance to the spinal column, the influence of the peripheral part of the conducting pathway could be neglected for STT CV calculation (Cruccu et al., 2000). In 15 subjects the stimulated locations were at intervals of approximately 20 cm, while in 5 subjects the intervals were approximately 5-10 cm. Subjects sat comfortably on a chair without leaning against the backrest. Stimulation intensity was adjusted individually for all locations, by using temperatures that were 3° C higher than the pain threshold, to induce reliable but tolerable pain (Bromm and Lorenz, 1998). The pain threshold obtained before the main experiment was defined as the average of the first or last painfully perceived temperature pulses in series of ascending and descending temperatures. During the main part, the four locations were stimulated in turn. This sequence was repeated 30 times. The first location and the direction of stimulation (up- or downwards) were balanced across subjects. The inter-stimulus interval was 10-12 s, totaling approximately 45 s for a single location. Thermode placement was varied within an area of approximately 15 cm^2 for each location.

Subjects had to rate the pinprick pain intensity of each stimulus 4 s after stimulation upon an acoustic signal on a scale ranging from 0 to 10, where 0 meant no pain and 10 the worst imaginable pain. This task ensured constant attention within and among subjects and the delay in pain rating prevented the affection of late pain evoked potential components by the subsequent motor preparation. Furthermore subjects were asked not to blink more than necessary and especially not in response to the painful stimuli.

2.3.3 EEG measurement and analysis

Continuous EEG was sampled at 500 Hz and recorded with 30 scalp electrodes and two electrodes below the outer canthus of each eye with the average reference as the recording reference (QuickAmp, Brainproducts, Munich, Germany). Subject ground was at the AFz position. For 5 subjects active Ag/AgCl electrodes (actiCAP, Brainproducts, Munich, Germany) and for 15 subjects passive Ag/AgCl ring electrodes were applied. No differences in EEG parameters were found between the two systems, therefore all data was pooled for the analyses. Impedances were kept below 20 kΩ (Ferree et al., 2001). The EEG analyses were done using Brainvision Analyzer software (Brainproducts, Munich, Germany). Raw data was filtered offline from 0.5-30 Hz. An independent component analysis was performed in order to remove eye blink (Halder et al., 2007; Halder et al., 2006; Jung et al., 2000) and stimulus-locked artifacts (Iannetti et al., 2006). Segmentation was conducted according to stimulus onset from -100 ms to 1000 ms that was written online as a marker into the EEG when the temperature started to rise. Trials which elicited no painful pinprick (pain rating=0 or missing) or with artifacts that exceeded ±80 µV were automatically excluded from all analyses, except for the analysis of pain ratings. Averages were calculated for every single subject and location, whereby only averages with more than 15 remaining sweeps were included in the subsequent analysis (see Tab. 2.1 for the number of averages per condition). The pain detection rate was defined as the number of painfully perceived stimuli over the total number of segments that were not previously excluded due to artifacts.

In order to automatically detect the latencies of the most prominent and commonly investigated P2 component (Kakigi et al., 2005) we used a method based on topographical information. This method is particularly useful to identify the latency of identical cortical processes in different subjects and to subsequently estimate the sources underlying a specific scalp topography (for a review see (Michel et al., 2004)). All averaged evoked potentials of every subject and stimulated location were entered into a spatial cluster analysis and the number of distinct microstates was determined according to a cross-validation criterion (Pascual-Marqui et al., 1995). This procedure identifies stable map topographies that consistently occur (Halder et al., 2005; Michel et al., 2001; Stein et al., 2006). The microstate explaining most of the variance corresponded well to the expected P2 topography (Bromm and Treede,

1991; Treede et al., 1988) and was therefore selected (see Fig. 2.4B). Subsequently, the covariance of this topographical map with the averaged evoked potentials at every single location was calculated for all subjects separately (Brandeis et al., 1992). The maximal covariance within a detection window (350-600 ms after stimulus onset for the points on the back and 450-700 ms for the ankle) that was chosen in the grand mean of all subjects was defined as the P2 latency. After visual inspection, we manually shifted the peaks in one subject that exhibited a dual covariance peak and where the automated procedure selected the second peak instead of the first. As for clinical applications such a procedure is not suitable, we also automatically detected the peaks at the Cz electrode, where maximal P2 activity is usually determined. The same detection windows were chosen as for the topographical analysis and a correlation between the latencies obtained with the two methods was performed. Finally, we also determined the latencies of the N2 component (Bromm and Treede, 1991; Kakigi et al., 2005; Treede et al., 1988), identified as the maximal negative Cz deflection in a time window of 200-550 ms for all locations.

For the grand average over all subjects after stimulation at the upmost location on the back, the global field power (GFP) and the global map dissimilarity (GMD) were calculated. GFP indicates the overall field strength and is calculated as the spatial standard deviation over all electrode potentials at every time point. GMD is a measure of topographic instability indicating topographic changes and is calculated as the difference between the current and the preceding map scaled to GFP for abolishing influence of the field strength (Brandeis et al., 1992; Brandeis et al., 1998).

The STT CV was calculated as the reciprocal slope of the regression line of the latencies (for P2: microstate and positive Cz peaks, for N2: negative Cz peaks) for back stimulation against distances to the brain (Cruccu et al., 2000). One subject was excluded from this analysis, due to very low pain thresholds (41.8° C, 38.5° C, 38.3° C, 41.8° C for the three locations at the back and ankle, respectively) and rather long latencies. We assumed that the small and considerably delayed visible potentials could be delivered by more slowly conducting C-fibers (Kakigi et al., 2005).

The peripheral CV was calculated by dividing the microstate P2 latency difference of stimulation at the lower back (T10 dermatome) and at the ankle by the distance between the two locations. As the afferent nerves originating at the ankle enter the spine at L4/5, that is 6 segments lower than T10, the calculation yields a result mixed

of a peripheral and a considerably smaller central portion. Therefore, this calculation serves as an approximation for the peripheral CV. Due to non-recordable potentials after ankle stimulation or stimulation at the back above the level of T10 only 9 subjects were included in this analysis.

2.3.4 Cortical source estimation

Cortical sources best explaining the P2 topography were determined by standardized low resolution brain electromagnetic tomography (sLORETA) (Pascual-Marqui, 2002). Topographies of the P2 component of every subject at every location were transformed into activity of 6239 grey matter voxels (xyz-values) without applying regularization. Eye electrodes were previously excluded (Gottselig et al., 2004). A paired t-test against zero was calculated to find consistently activated cortical sources. SLORETA results are reported in the Montreal Neurological Institute (MNI) coordinate system. Significance level was set to $P<0.01$.

2.3.5 Statistics

Linear regression analyses were conducted to investigate the dependence of latencies from pain thresholds and distances to the brain. These analyses were performed for the P2 peaks determined by the microstate analysis, as well as for those identified by conventional peak detection at the Cz electrode (N2 and P2). Repeated measures analysis of variance (ANOVA) was used to investigate differences between the thresholds and pain ratings at the four different stimulation locations. A paired t-test of individual CVs against 0 was conducted to test for statistical significance of the peripheral CV. Finally, a correlation analysis was used to compare the two approaches for peak determination for the P2 component as well as for a comparison between the N2 and the P2 peaks, identified at the Cz electrode. The significance level was $P<0.05$.

2.4 Results

2.4.1 Thresholds, pain ratings and distances

ANOVA showed a significant main effect of location on thresholds ($F(3,9)=6.588$, $P<0.05$). Post-hoc t-tests revealed significant differences between the three thresholds on the back and the one at the ankle ($P<0.05$ for all three pairs) whereas thresholds at the back were not significantly different from each other. ANOVA

showed no effect of location on the pain detection rates (F(3,9)=0.33, P=0.80). For the pain ratings no significant main effect of location could be detected (F(3,9)=1.19, P=0.37). One subject did not rate one stimulation at back 1 and another subject did not rate seven stimulations at the ankle. The corresponding segments were excluded from all analyses. See Tab. 2.1 for mean thresholds, distances, detection rates and pain ratings (0-10).

	N	Initial latency [ms]	Normalized latency [ms]	Threshold [°C]	Pain detection rate	Pain rating (0-10)	Distance to inion [m]	Detection window P2 [ms]
back 1	19	489.5+-52.2	364.6+-31.7	47.1+-3.7	0.86+-0.18	1.71+-1.0	0.22+-0.08	350-600
back 2	19	489.8+-47.6	377.0+-39.4	45.9+-3.1	0.89+-0.13	1.68+-1.0	0.39+-0.10	350-600
back 3	18	506.6+-52.7	397.3+-51.9	45.5+-3.8	0.87+-0.15	1.76+-1.0	0.52+-0.13	350-600
ankle	13	596.6+-60.2	452.1+-53.3	48.9+-3.9	0.76+-0.25	1.40+-0.5	1.52+-0.09	450-700

Tab. 2.1: Mean and standard deviation (SD) of parameters of interest.

2.4.2 Waveforms of the evoked potentials

In the grand averages for the single locations the three mainly described pain evoked potential components (Bromm et al., 1991; Kakigi et al., 2005) were visible (shown for stimulation at back 1 in Fig. 2.1). The peaks of GFP correspond to the negative (N2) and positive (P2) Cz peaks separated by a maximum in GMD. According to the literature, the N1 peak preceding N2 was determined at the early minimum of the contralateral T8 electrode (Bromm et al., 1991; Treede et al., 1988). However, N1 was rarely visible in single subjects.

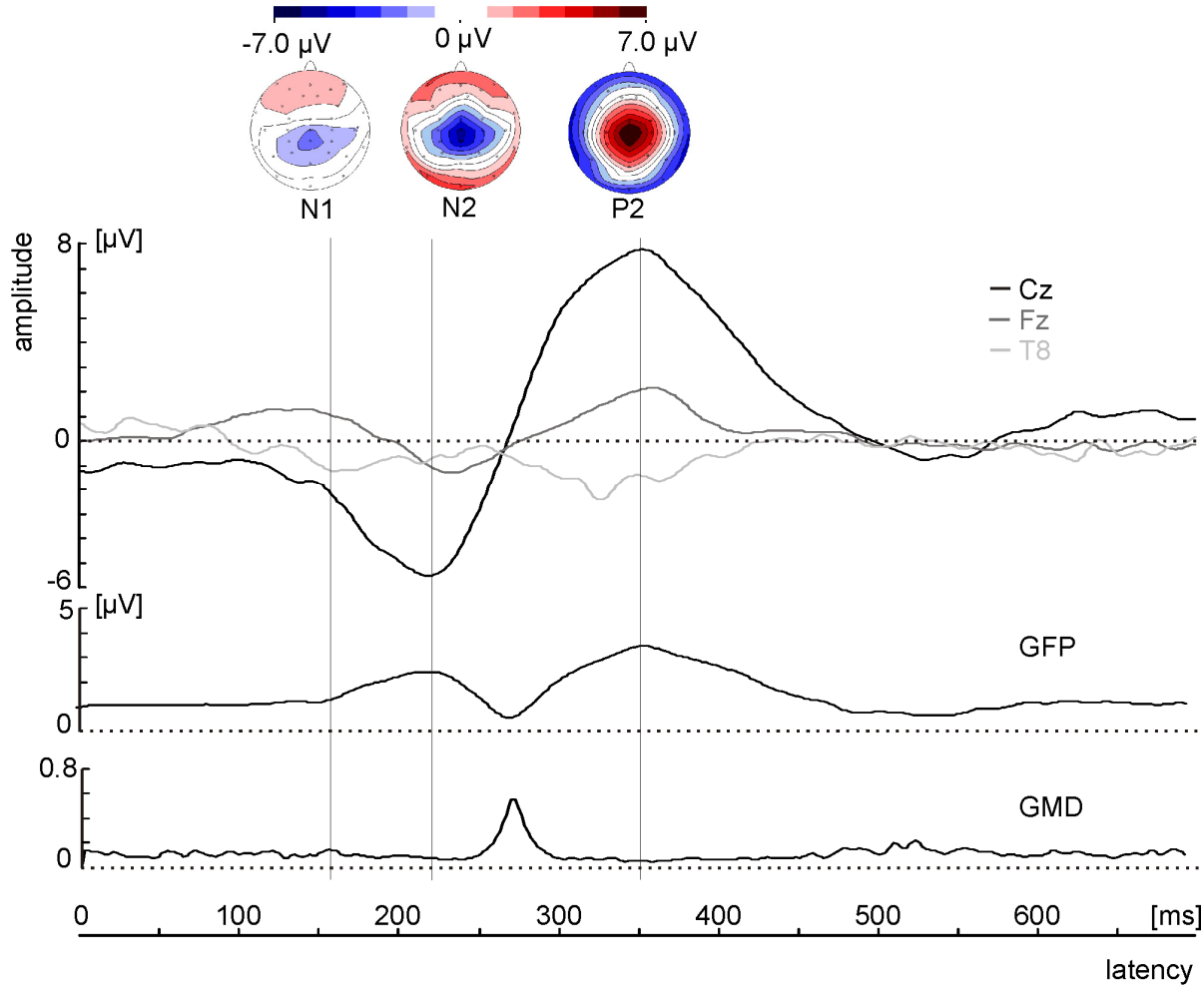

Fig. 2.1: CHEP after stimulation at the upmost location on the back. All latencies are normalized for pain thresholds. (Upper part) Grand average of all subjects. Cz (black), Fz (interpolation using FCz and Fpz; dark grey), T8 (light grey), all potentials are shown against average reference. (Middle part) Global field power (GFP). (Lower part) Global map dissimilarity (GMD). The main CHEP components are shown as topographies. N2 and P2 at the negative and positive peaks of Cz and corresponding positive peaks of GFP, N1 at the minimum of T8 preceding N2 (temporal electrode contralateral to stimulation). Negativity downwards.

2.4.3 Latencies and conduction velocity estimates

Linear regression analysis of initial P2 peak latencies (identified by the microstate procedure) from all stimulation locations in all subjects against distances to the brain showed only a significant relationship if all four locations were included (r^2=0.372, B =84.352, F=39.707, P<0.001) but not if only the initial latencies after back stimulation were considered (r^2=0.012, B=34.541, F=0.636, P=0.429; see Fig. 2.3). A significant linear relationship of the P2 latencies from all stimulation locations in all subjects

against the respective thresholds was found (r^2 = 0.337, B =10.363, F=34.0; P<0.001; see Fig. 2.2).

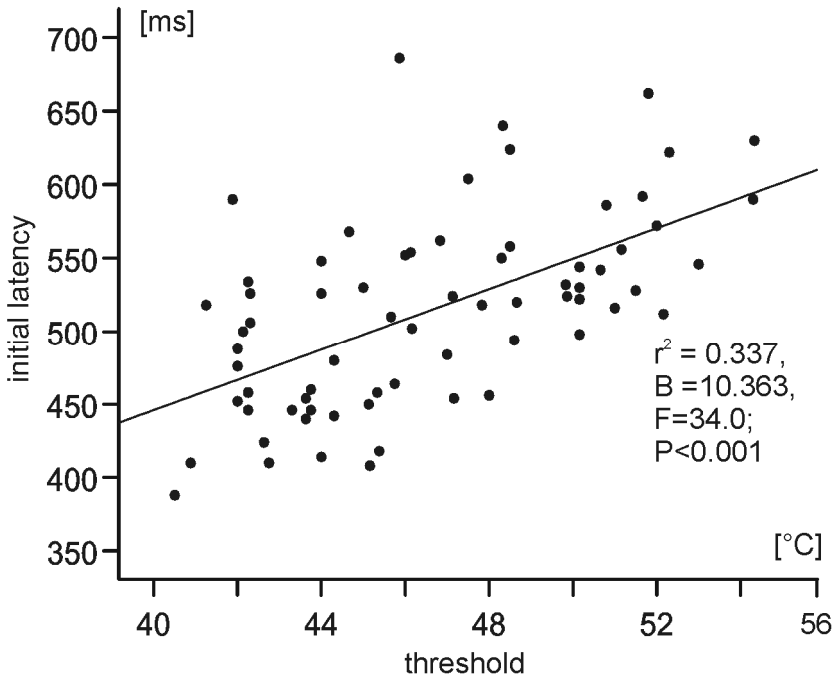

Fig. 2.2: Regression between latencies and pain thresholds after stimulation at all locations.

As latencies were expected to be dependent on distance, a partial correlation analysis, controlling for distances to the brain was performed and still revealed a significant correlation (r^2=0.329, P<0.001). Therefore, we normalized the latencies using the slope B of the regression line of latencies vs. thresholds to a hypothetical threshold of 35° C, which was the baseline temperature. This normalization abolished the influence of thresholds and thus made the latencies comparable between and within subjects. In addition, normalizing to the baseline temperature should draw our latencies closer to results obtained in laser studies, where threshold temperature is reached immediately. Linear regression analysis of the normalized latencies after back stimulation at all locations on the back in all subjects and the distances to the brain showed a significant relationship (r^2=0.103, B=89.3, F=6.215, P<0.05) yielding a STT CV of 11.2 m/s, see Fig. 2.2. Mean normalized and initial latencies are reported in Tab. 2.1.

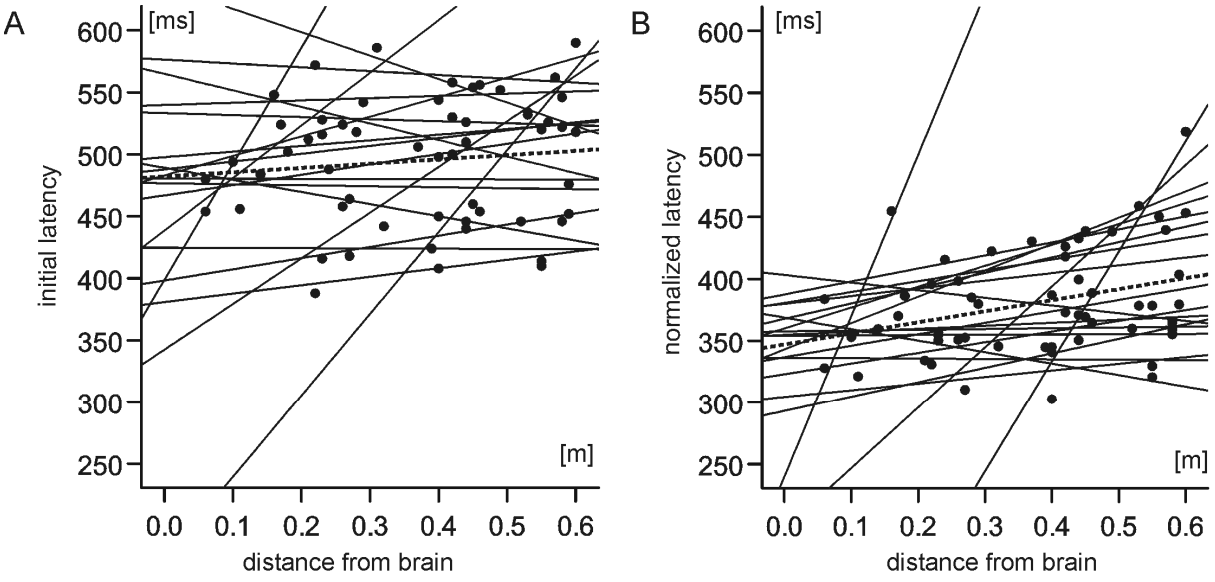

Fig. 2.3: Latency vs. distance from stimulation location on the back to the brain; bold line: Total regression of all included points in all subjects (bold). Regression lines for individual subjects (thin line). (A) Initial latencies. (B) Normalized latencies.

The P2 peaks determined using maximal positive Cz activity correlated significantly with the microstate peaks (Pearson correlation: back 1: r =0.82, P<0.001; back 2: r = 0.83, P<0.001; back 3: r=0.72, P<0.005, ankle: r=0.60, P<0.05). The equivalent regression analysis latencies vs. thresholds was significant (r^2=0.405, B =11.074, F=45.6; P<0.001) and the STT CV after normalization for the thresholds was 13.4 m/s (r^2=0.071, B=74.4, F=4.113, P<0.05). One of the P2 Cz peaks had to be manually shifted as it was just outside the detection window.

When analyzing the N2 peak, as assessed by maximal negative deflection at the Cz electrode, three additional subjects had to be excluded from analyses involving the ankle condition, as no N2 component could be identified. Thus, only their N2 latencies after back stimulation were included in the analysis. The N2 peaks correlated significantly with the P2 peaks (both determined at the Cz electrode; Pearson correlation: back 1: r=0.62, P<0.005; back 2: r=0.69, P<0.005; back 3: r=0.53, P<0.05, ankle: r=0.62, P<0.05). The regression analysis between N2 latencies and thresholds was significant (r^2=0.463, B =11.237, F=56.1; P<0.001) and the STT CV after normalization for thresholds was 13.3 m/s (r^2=0.106, B =75.217, F=6.391; P<0.05).

The peripheral velocity estimate, calculated using P2 latencies obtained by microstate analysis, was 18.3 ± 13.8 m/s (mean+-SD) (t=3.968, P<0.01).

2.4.4 Cortical source estimation

Cortical activity explaining the P2 topography was found bilaterally in the posterior cingulate/cingulate gyrus, in the insula ipsilateral to stimulation and in the ipsilateral parahippocampal gyrus, see Fig. 2.4. The threshold for significance at a level of P=0.01 for the two-tailed t-test was computed as 4.7.

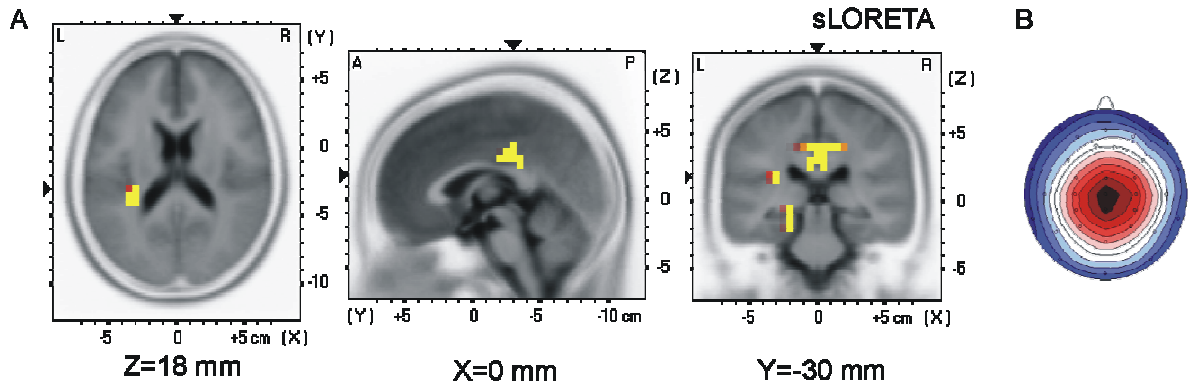

Fig. 2.4: A Most significant cortical activity estimated over all subjects and locations at the microstate P2 peak. See also corresponding Tab. 2.2 **B** Topography used to determine the P2 microstate peak.

Region	Maximal t-value	Brodman Area	MNI coordinates of maximum		
Cingulate Gyrus / Posterior Cingulate	15.18	23	-5	-30	25
	15.06	23	5	-30	25
Insula	15.09	13	-30	-40	20
Parahippocampal gyrus	14.72	28	-20	-25	-10

see also corresponding Fig. 2.4

Tab. 2.2: Main cortical sources of P2

2.5 Discussion

It was the aim of this study to determine the central CV of Aδ-fibers of the human pain pathways using contact heat stimulation, thereby replicating results previously reported using the more established method of laser stimulation (Treede et al., 2003). We expect that contact heat could be more widely applied in clinical settings than laser stimulators, due to less restrictive safety precautions (e.g. no need for fulfilling local laser safety guidelines and approved measuring rooms). This study could therefore provide the basis for future clinical applications. Through the stimulation alongside the backbone, the peripheral part of the conducting pathway is

held constant and can therefore be neglected to estimate the CV of the STT (Cruccu et al., 2000). The resulting STT CV of 11.2-13.4 m/s is in the range of previously reported estimates after laser stimulation (Cruccu et al., 2000; Iannetti et al., 2003; Kakigi et al., 1991; Rossi et al., 2000). The peripheral CV was at 18.3 m/s slightly higher than the central CV, but still in the range of Aδ-fiber CV (Kakigi et al., 2005).

The highly significant dependence of the initial latencies on the pain thresholds, even when controlled for the influence of distance, as well as the absent relation between latencies and distances indicate that the influence of the threshold on latencies needs to be considered for STT CV estimations. Only if this dependence is removed, are the STT CV estimates and the latency comparisons significant and in accordance with previous studies using laser stimulation. In order to confirm these findings, we additionally analyzed the N2 component that precedes P2 and found the same dependency of latencies on thresholds. This influence becomes more important if stimulated points are close as in our case the points at the back. We hypothesize that the low heating rate is responsible for the thresholds' effect, as nociceptors are not activated at the beginning of the stimulus but during the heat ramp (Baumgartner et al., 2005). This suggestion is supported by the similar gradients of the temperature curve (nominally 70°C/s) and the reciprocal slope of the regression latencies against thresholds (Fig. 2.2, 96.5° C/s). For laser devices with considerably higher heating rates this effect seems negligible. It is important to note that the effect of the varying thresholds on latencies would not have shown up in the grand mean, because thresholds did not systematically vary across the stimulated regions on the back. It only became important for minimizing variance within and across subjects, resulting in more robust and significant statistics. As shown in Fig. 2.5 the normalization procedure leads to higher amplitudes in the grand mean over all subjects, indicating that variance across subjects is minimized.

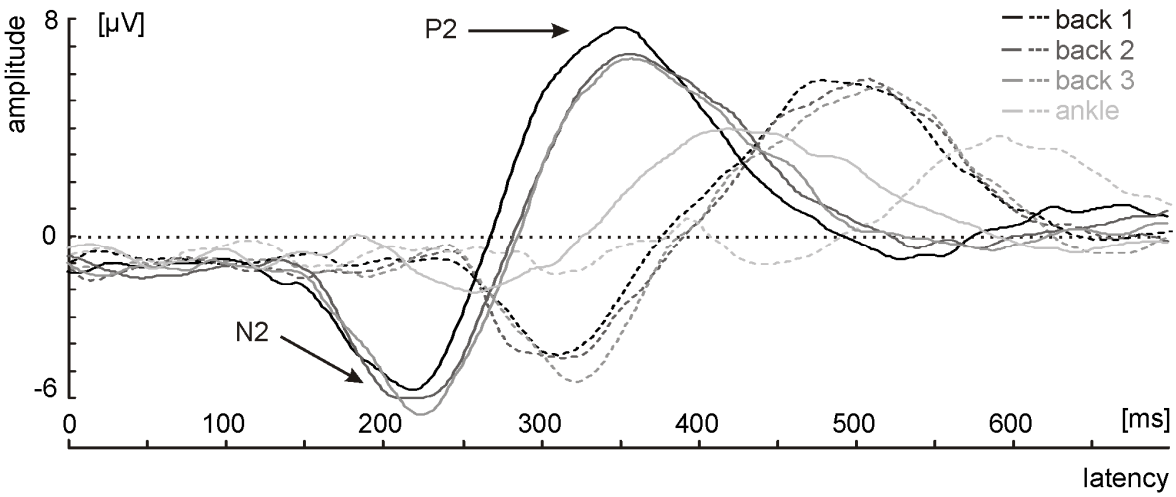

Fig. 2.5: Cz traces of the grand average over all subjects for each stimulation location. Dashed lines represent initial potentials; continuous lines represent potentials normalized for pain thresholds. Note the enhanced amplitudes after the normalization. Negativity downwards

However, individual CV estimates still show some variation and as visible in Fig. 2.3, are not meaningful for every single subject. Most of the outliers were stimulated at points very close together. It is therefore important to stimulate points which lie approximately 20 cm apart. Another source of variability could be found in the applied method of threshold determination since several subjects reported difficulties to judge differences between warmth sensation and pain. For future studies it is therefore important to rely on a robust method for threshold determination. As a consequence of the STT CV variability in healthy subjects, it remains unclear, whether STT CV as estimated in this study by regression analyses, is useful to differentiate between individuals with intact or damaged STT. As shown in Fig. 2.6, the latency increase in a patient suffering from SCI is substantial. Therefore, it might be sufficient to compare CHEP latencies after stimulation at a given location instead of relying on STT CV estimation. However, further studies including patients will be needed to judge what parameters are useful for assessing STT damage after contact heat stimulation.

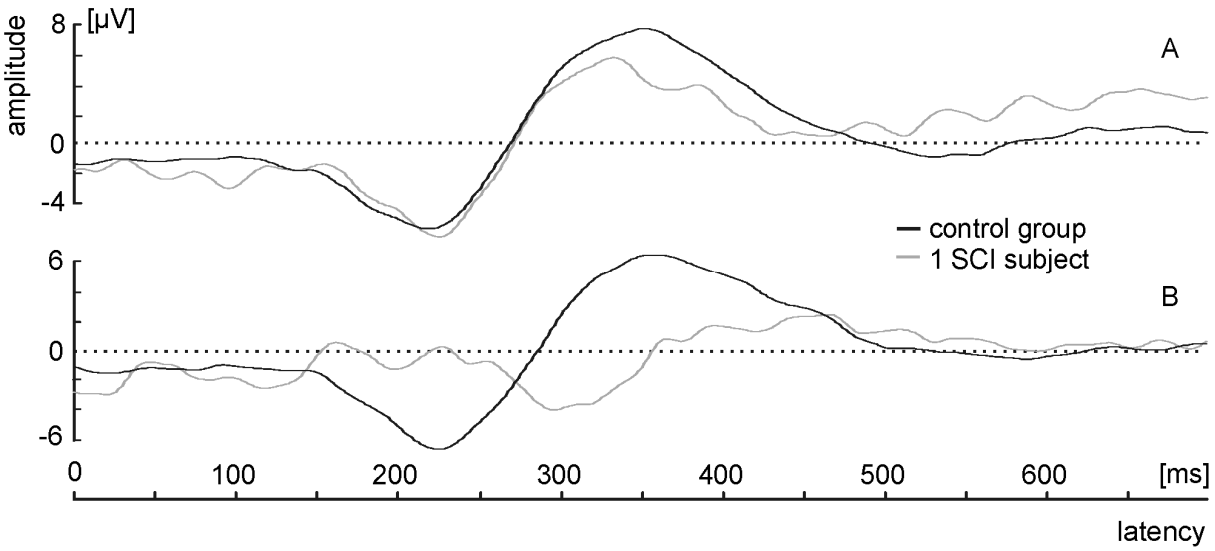

Fig. 2.6: Comparison of latencies from all healthy subjects (black) and one spinal cord injured subject (SCI) (gray). All latencies were normalized for pain thresholds. **A** Contact heat stimulation at back 1 (healthy subjects) and three dermatomal segments above the neurological lesion level (one SCI subject). **B** Stimulation at back 3 (healthy) and three segments below the neurological lesion level (SCI). Latencies of the SCI subject were normalized to the mean distances of the locations back 1 and back 3 of the healthy subjects using a STT CV of 11.2 m/s. Negativity downwards. The spinal cord lesion was at a level of T8 and the subject exhibited a zone of partial preservation in which the stimulation below the lesion level (T11) was applied. Below L2 CHEP and any sensation upon contact heat were totally extinguished.

The use of longer distances between stimulation locations, stimulation of single points only or assessment of grand means and not individual CVs might explain why the influence of the thresholds on CHEP was not detected in earlier studies (Chen et al., 2001; Granovsky et al., 2005). Another reason might be found in the adapted method using pulsed stimuli for pain threshold determination that we applied. We considered it important to assess the pain threshold with the same stimulation that was later applied to measure CHEP. It could be argued that what we assessed as the pain threshold might be a mixture of the threshold temperature for nociceptor activation and the heat conductance profile of the skin at a given location (Arendt-Nielsen and Chen, 2003).

It is noteworthy that we stimulated in accordance to the pain threshold, therefore, using the constant heat ramp, higher temperatures inevitably led to longer stimulation times. It was thus impossible to exclude that longer stimulation times rather than higher thresholds were responsible for the longer latencies with higher thresholds. We therefore conducted some follow-up measurements including 16 subjects with

two different stimulation temperatures applied at the same location. The results comparing stimulation at pain threshold +2° C and threshold +4° C indicate that both, N2 and P2 latencies, are not affected by stimulation temperature (paired t-tests, N2: t = -0.15 P = 0.88, t = -0.81, P = 0.43). This indicates that indeed thresholds and not stimulation duration (and correspondingly different temperatures) are responsible for the observed effect.

The normalization of the latencies to the baseline temperature of 35° C drew the latencies closer to the previously published values obtained with CO_2 laser stimulation, which ranged between 290-320 ms (Cruccu et al., 2000; Iannetti et al., 2003) and 320-360 ms (Qiu et al., 2001) for stimulation on the highest and lowest location on the back, respectively. Nevertheless, the normalized latencies are still considerably longer (about 50 ms) than the previously published values. One possible explanation could be that lasers penetrate into the skin and therefore activate nociceptors directly while contact heat spreads passively from the skin to the nociceptors and activates them successively. This has been suggested previously by a study directly comparing contact heat and laser evoked potential latencies for stimulation in the trigeminal territory (Truini et al., 2007), where the latency difference between the two methods amounted to 100 ms.

In contrast to earlier studies where the P2 peak was determined at the Cz electrode (Bromm and Lorenz, 1998; Kakigi et al., 2005), we relied on an automated procedure that was based on topographical information. The topographical analysis does not only rely on the potential recorded at the vertex electrode Cz, but it considers a more complete picture by assessing the potential on the whole scalp. However, several factors indicate that a Cz derivation is sufficient for the accurate estimation of the STT CV, either for N2 or P2: the correlation between peaks determined by Cz and the topographical analysis, the correlation between N2 and P2 peak latencies, as well as comparable resulting CVs.

In general, the N2 and P2 components were reliably detected in most of the subjects. However, after stimulation at the ankle it was impossible in 6 subjects to obtain 15 painful out of 30 heat applications, which was the minimal amount needed to be included in the analysis. This occurred even though we adapted the stimulation temperature to the pain threshold at all locations individually. This might indicate that the adaptation was stronger at the ankle than at the back. Furthermore the lower

nociceptor density at the ankle (Agostino et al., 2000) and the dispersion along the longer distance to the brain led to reduced amplitudes of the evoked potentials. In contrast to laser stimulation, where potentials can be absent only after ankle stimulation in elderly subjects (Truini et al., 2005), contact heat stimulation at the ankle does not elicit reliable potentials, even in young subjects.

In contrast to the most prominent N2 and P2 components, the N1 component was hardly detectable in single subjects, although visible in the grand average over all subjects, see Fig. 2.1. Of note, neither a minimum in GFP nor a peak in GMD is visible between the putative N1 and the subsequent N2 component, indicating that those components are difficult to separate. The use of N1 after contact heat stimulation for assessing STT damage seems therefore questionable.

The brain regions involved in the generation of the P2 component, see Fig. 2.4, were estimated to be located in a cluster covering parts of the cingulate gyri bilaterally, in the ipsilateral insula and in the ipsilateral parahippocampal gyrus. Unlike other studies using dipole models for determining brain sources after painful stimulation (Valeriani et al., 2002; Valeriani et al., 1996), the sLORETA method applied in this study involved no a priori knowledge about the number of activated regions. The only assumption made is that nearby cortical regions exhibit similar activity, thus the “smoothest” possible source solution is calculated (Pascual-Marqui, 2002). However, a possible drawback of the method could be that nearby simultaneously active sources cannot be separated (Wagner et al., 2004). The cingulate gyrus and the insula are commonly found to be active during pain processing (Apkarian et al., 2005; Bentley et al., 2003; Bromm, 2004; Chen et al., 2002; Iannetti et al., 2003; Peyron et al., 2000). In earlier studies not only the ipsilateral but also the insula contralateral to stimulation was activated. However, as we only investigated the late P2 component it is likely that the contralateral insula was activated earlier.

Assessing the integrity of the STT in SCI has been postulated to offer most indications for laser and contact heat stimulation (Treede, 2003). Together with the established assessments of the somatosensory and motor tracts (Curt and Dietz, 1997; Curt et al., 1998) and the recently suggested assessment of the vestibulospinal tract by galvanic vestibular stimulation (Wydenkeller et al., 2006), CHEP could be used to describe a spinal lesion in detail.

In conclusion, this study shows that the CV of the central pain pathways can be assessed using CHEP if the influence of the slow heat ramp is considered by controlling for pain thresholds.

3 Study 2: Heat transfer model confirms influence of pain threshold on latency of contact heat evoked potentials[2]

3.1 Abstract

Objective

To clarify the potential influence of the factors thermode application force and stimulation temperature on the contact heat evoked potential (CHEP) read-out parameters latency and amplitude.

Methods

The influence of stimulation temperature and thermode application force was investigated using a heat transfer model based on the bioheat equation of Pennes. The modeled effect of differing heat pain thresholds on CHEP latencies was compared to the corresponding dependence determined in an earlier study. For model validation, thermode application force and stimulation temperature were also investigated experimentally.

Results

The influence of the pain threshold on CHEP latency was confirmed by the heat transfer model, while the influence of thermode application force was small in both the experiment and the modeling. The N2 was more stable than the P2 CHEP component for stimulation with different temperatures above pain threshold.

Conclusions

Due to the low heating rate of the contact heat stimulator and the passive heat spread in the skin, the pain threshold has a considerable influence on the CHEP latency. In contrast the thermode application pressure might be neglectable for studies investigating CHEP latency.

[2] This manuscript is under construction and will be submitted to the journal: Clinical Neurophysiology. The authors were Susanne Wydenkeller, Manuela Tobler, Regula Wirz, Vartan Kurtcuoglu, Dimos Poulikakos and Pascal Halder. The measurements were conducted by Regula Wirz. Data were analysed by Susanne Wydenkeller and Regula Wirz. The heat transfer modell was provided by Manuela Tobler, supervised by Vartan Kurtcuoglu and Dimos Poulikakos. The manuscript was written by Susanne Wydenkeller and revised by the co-authors.

3.2 Introduction

Contact heat evoked potentials (CHEP) are increasingly used to objectively study small nerve fiber function and pain processing both in health and disease (Chen et al., 2001). Especially decreased amplitudes and increased latencies are thought to be indicative of dysfunctional pain pathways (Atherton et al., 2007; Chao et al., 2008; Iannetti et al., 2006; Truini et al., 2007; Valeriani et al., 2002; Wydenkeller et al., 2008). Consequently, factors that potentially influence these CHEP read-out parameters other than dysfunction in the involved pathways should be investigated. A previous study has shown that the pain threshold positively correlates with the latency of the CHEP N2 and P2 components (Wydenkeller et al., 2008). It has been hypothesized that the relatively slow heating rate of contact heat stimulation in combination with the passive conductance of the heat from skin surface to nociceptors is responsible for this interdependence. A model of the passive heat transfer from skin surface to the nociceptors could substantiate these experimental findings. Among a broad range of models, the Pennes bioheat equation (Pennes, 1948) is one of the simplest and most widely applied (for a review see (Wissler, 1998)). In this model, it is assumed that metabolic heating, the perfusion rate and thermal conductivity are uniform within the different tissue layers. In addition, the heat source is assumed to be isotropic.

Another hypothetical source of CHEP latency variation is the force with which the thermode is applied on the skin. More applied force could lead to better skin contact of the thermode and thus better heat transfer and shorter CHEP latencies. While this was repeatedly hypothesized (Iannetti et al., 2006; Plaghki and Mouraux, 2003), it was never investigated thoroughly. As pain ratings correlate to the amplitude of painful evoked potentials (Carmon et al., 1976; Garcia-Larrea et al., 1997; Granovsky et al., 2007; Greffrath et al., 2007) it is expected that the thermode application force modulates CHEP amplitude. It is conceivable that the thermode application force mainly alters the heat transfer from the thermode to the skin by providing better contact. Direct measurement of this temperature is difficult as the measurement device influences the results. Conversely, the heat transfer model can be applied to investigate it on a theoretical basis. Modeled effects and CHEP changes obtained after contact heat stimulation at systematically varied thermode application force can then be compared.

It was the aim of this study to clarify the relation between CHEP latency and heat pain threshold with a theoretical heat transfer model. Additionally, the model served to investigate the influence of thermode application force on CHEP latency and amplitude as well as pain ratings. Both the parameters stimulation temperature and thermode application force were in addition investigated experimentally and compared to the results of the modeling.

3.3 Methods

3.3.1 Contact heat evoked potentials

All measurements were approved by the local ethics committee and conducted in accordance with the declaration of Helsinki. Informed consent was obtained from all subjects before the experiment. Fourteen healthy subjects (age: 26.9±9.2 years, mean±s.d., 8 women) were included in the experiment on varying stimulation temperature (ExpA). Out of these, 12 participated also in the experiment on varying thermode application force (ExpB; age: 27.9±10 years, 6 women). The 2 experiments were conducted in different sessions, separated by at least 5 months.

Painful heat stimuli were applied to the skin of the back using a “Pathway” contact heat stimulator described elsewhere (Wydenkeller et al., 2008) (Medoc, Ramat Yishai, Israel). Subjects sat comfortably on a chair and the thermode was fixed to the back with an elastic strap for ExpA. During ExpB subjects lay face down on an examination couch and the thermode, loaded with different weights (0 g, 120 g, and 500 g), was positioned on the back. The net weight of the thermode head was 180 g. Thus the resulting thermode application forces were 180 g, 300g and 680 g. Heat pain thresholds were determined before the experiments. Subsequently, stimulation temperature was set 3° C higher in ExpB. In ExpA three temperatures in relation to the pain threshold (Thr) were investigated. Thr+0° C, Thr+2° C or Thr+4° C. The stimulation temperature never exceeded 55° C and the inter-stimulus interval was 14-17 s. Twenty (ExpB) to thirty (ExpA) stimuli were applied block-wise for every temperature or application force. Within one experiment the order of the blocks was pseudo-randomized. Subjects had to rate the pain intensity prompted by an acoustic signal 4 s after each stimulation. A numeric rating scale from 0 to 10, where 0 meant no pain and 10 the worst imaginable pain was used. Subjects were instructed to look straight ahead and not to blink more than necessary.

Continuous EEG was recorded at 500 Hz with 30 Ag/AgCl electrodes on the scalp and two electrodes below the outer canthus of each eye (Quickamp, Brainproducts GmbH, Germany). Subject ground was at the Afz position and the average of all electrodes was used as reference. Impedances were kept below 20 kΩ (Ferree et al., 2001; Halder et al., 2007). An optical isolation box (Brain Vision OptoBox, Brainproducts GmbH, Germany) was interposed to minimize artifacts. The data was filtered offline at 0.5-30 Hz and segmented from -100 ms to 1000 ms according to stimulus onset. Trials with artifacts exceeding ±80 µV were automatically excluded from all analyses. The minimum of segments included in the analysis was 15. In subjects with less segments included (n=2), an independent component analysis was performed to remove eye movement and blink artifacts (Jung et al., 2000).

CHEP components were defined as follows: N2 and P2 were the main negative and positive peaks on the Cz electrode. Latency and amplitude of the CHEP were determined in the averages of every subject in time windows N2 250-460 ms, P2 400-640 ms. The N2 and P2 latencies at Thr+2° C and Thr+4° C of some subjects were reported before (Wydenkeller et al., 2008).

The influence of thermode application force on heat pain thresholds was investigated in an additional experiment (ExpC) with 12 subjects. The thermode was positioned on the back with two different application forces: either with the net weight only (force=180 g) or loaded with 500 g (680 g). Order of application force was balanced across subjects.

3.3.2 Statistics

Non-parametric Friedman or paired Wilcoxon tests were used if data was not normally distributed according to Shapiro Wilk's test to test for differences between the different conditions (all latencies and amplitudes). Heat pain thresholds were normally distributed and thus compared with paired t-tests. The potential distribution at the time points of the N2 and P2 peaks (topographical maps) were tested for correspondence between the different conditions. For this, the respective topographical maps were compared with statistical non-parametric mapping paired t-statistics on subject-wise root mean square-normalized data (Nichols and Holmes, 2002; Pascual-Marqui, 2002). The level of significance was set to 0.05 for all tests.

3.3.3 Heat transfer model

The Pennes heat transfer model was investigated in one dimension. The tissue underlying the thermode was modeled as skin, fat and muscle. An additional layer of 20 µm strength was introduced on the tissue surface to account for the gap between stimulator and skin consisting of hair, sweat and air, see Fig. 3.1.

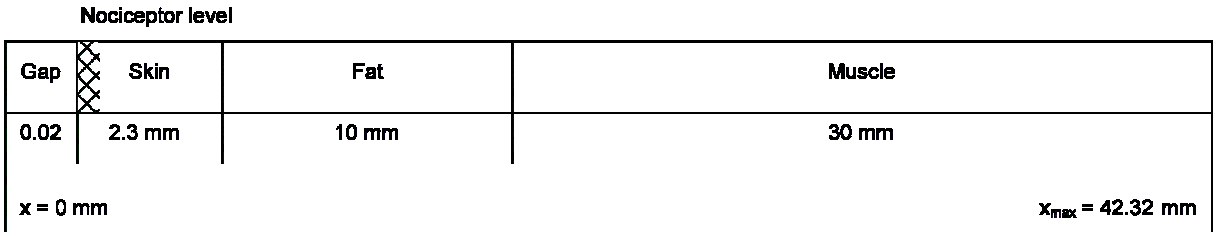

Fig. 3.1: Tissue model consisting of the four layers gap between thermode and skin, skin, fat and muscle. Nociceptors lie just beneath the skin surface in the skin layer. The extent of the skin layer was defined according to (Simonen et al., 1997).

As the skin on the back is relatively thick (1-2 cm), no large arteries or bones need to be considered. The bioheat equation of Pennes (Pennes, 1948) was simplified by neglecting metabolic heating and assuming thermal equilibrium between venous blood and tissue. Thus, Eq. 3.1 was used for the model where ρ, cp and k were the effective density, specific heat and thermal conductivity of the tissue (t) and blood (b), respectively. Ta is the arterial blood temperature, T the tissue temperature and $\dot{V}'''$ the perfusion rate. The tissue properties used here, Tab. 3.1 were proposed by Werner and Buse (1988).

$$\left(\rho c_p\right)_t \frac{\partial T}{\partial t} = \frac{\partial}{\partial x}\left(k \frac{\partial T}{\partial x}\right) + \left(\rho c_p\right)_b \dot{V}'''\left(T_a - T\right) \quad \text{Eq.3.1}$$

The geometry described in Fig. 3.1 was used to solve Eq. 3.1 from the beginning of the gap layer (x=0) to the end of the muscle layer (x_xmax). A linearly increasing temperature from 35° C at skin surface to 37° C inside the body was set as initial condition. Boundary conditions were the thermode temperature at the surface and a constant temperature of 37° C at the end of the muscle layer. The penetration of the heat stimulus into the skin was calculated for a nominal thermode destination temperature of 54° C. The input temperature was extracted from recordings provided by the Pathway stimulator analogue output of stimulation pulses with 35° C baseline and 70°C/s nominal heating rate. A mean nociceptor depth of 200 µm was assumed

(Novotny and Gommert-Novotny, 1988; Stoll and Greene, 1959; Tillman et al., 1995). The time between start of thermode temperature rise and maximal temperature at nociceptor level was calculated as function of stimulation temperature for nominal stimulation pulses between 40-54° C. This latency was thought to be related to the time from thermode temperature rise to nociceptor activation for different heat pain thresholds. Modelling of different thermode application forces was done by assuming different extent of contact area and thus different conductivities of the gap layer. For the condition gap_skin (good contact) the gap layer parameters were defined according to skin without perfusion. For condition gap_skin/air (bad contact) the parameters consisted of the respective weighted values from skin (weighted as 0.75) and air (0.25) without perfusion, see Tab. 3.1.

	Description	unit	blood	skin	fat	muscle	gap _skin	gap _skin/air	air
ρ	Density	kg/m^3	1059	1085	920	1085	1085	814	1.205
c_p	Heat capacitance	J / (kg·°C)	3850	3680	2300	3800	3680	3011	1005
k	Thermal conductivity	W/ (m·°C)		0.47	0.21	0.51	0.47	0.36	0.03
$\dot{V}'''$	Perfusion rate	1/s		$3.6 \cdot 10^{-4}$	$7.6 \cdot 10^{-5}$	$5.4 \cdot 10^{-4}$			
T_a	Arterial temperature	°C	37						

Tab. 3.1: Parameters used in the different constituents of the model.

3.4 Results

3.4.1 Varying temperature

The pain threshold was 46.2±2.4° C. Visual inspection of the CHEP disclosed constant appearance of the N2 component but conspicuous changes in the later positive component, see Fig. 3.2. Thus, the main positive peak in the grand average for stimulation at Thr+0° C even lay outside the time window for peak detection. Topographical comparison of the peaks showed constant N2 topography over the different stimulation temperatures. Conversely, the topography of the positive component manifested a trend towards being different between stimulation at Thr+4° C and Thr+0° C (t=3.51, t=3.44 was significant at a level of P=0.1). Due to this inconsistency in the positive component, the corresponding latencies and amplitudes

were not further analyzed as different topographical maps indicate different underlying cortical processes, for a review see (Brandeis and Lehmann, 1986). One subject did not show a CHEP at the lower stimulation temperatures and was thus excluded from analysis. N2 latencies were not significantly different between the applied temperatures, although a trend towards shorter latency with higher stimulation temperature was detected (P=0.069). Post-hoc paired tests disclosed a trend towards shorter latencies in the higher stimulation temperature compared to stimulation at Thr+0° C (Thr+4° C P=0.096, Thr+2° C P=0.084). Amplitudes (P=0.010), see Fig. 3.2, and pain ratings (P=0.000, median Thr+0° / Thr+2° / Thr+4° C: 0.14 / 0.28 / 0.77) increased in parallel with stimulation temperature.

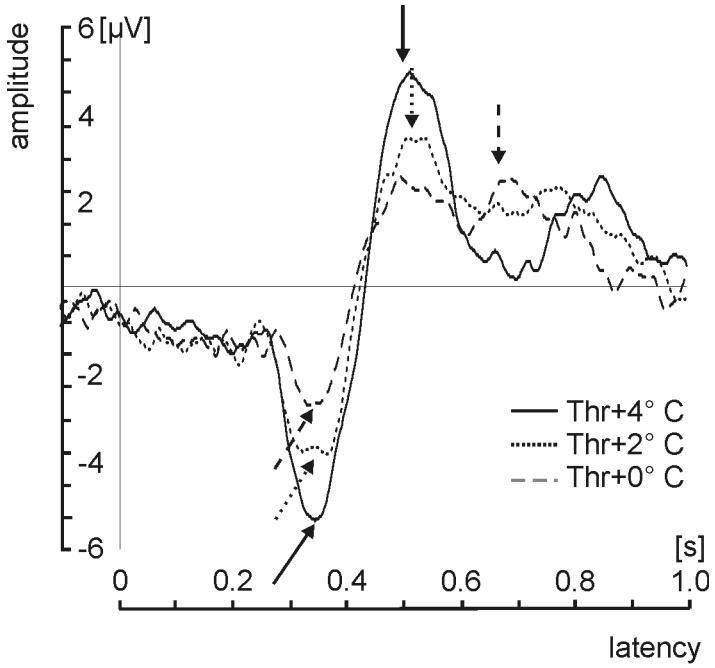

Fig. 3.2: Contact heat evoked potentials for different stimulation temperature on the vertex Cz electrode against average reference. Stimulation with pain threshold temperature (Thr) +0° C, Thr+2° C and Thr+4° C. Main positive and negative peaks are indicated with arrows. Note the consistency of the N2 latency for the different stimulation temperatures which is in contrast to variable positive peak.

3.4.2 Varying thermode application force

The pain threshold was 47.1±2.7° C. Upon visual inspection the CHEP consistently appeared in all conditions. Correspondingly, the comparison of peak topography showed constant N2 and P2 topographies over the different thermode application forces. CHEP latencies were not significantly different when the thermode was applied with different force (N2: P=0.42, P2: P=0.56). The same was the case for N2

amplitude (P=0.34) while the P2 amplitude was increased with higher force (P=0.046), see Fig. 3.3. Post-hoc paired tests disclosed that the effect on P2 amplitude was due to increased amplitude for the highest application force compared to the lowest (P=0.012). The pain rating was different between the three conditions (P=0.03) and tended to be lower for the condition where the least force was applied (median 180 g / 300 g / 680 g: 0.19 / 0.26 / 0.59; post-hoc paired tests 180 g vs. 300 g P=0.08, 300 g vs. 680 g P=0.29 and 180 g vs. 680 g P=0.01). Heat pain thresholds did not differ between the conditions in ExpC (180 g 47.8° C, 680 g 47.0° C, P=0.357).

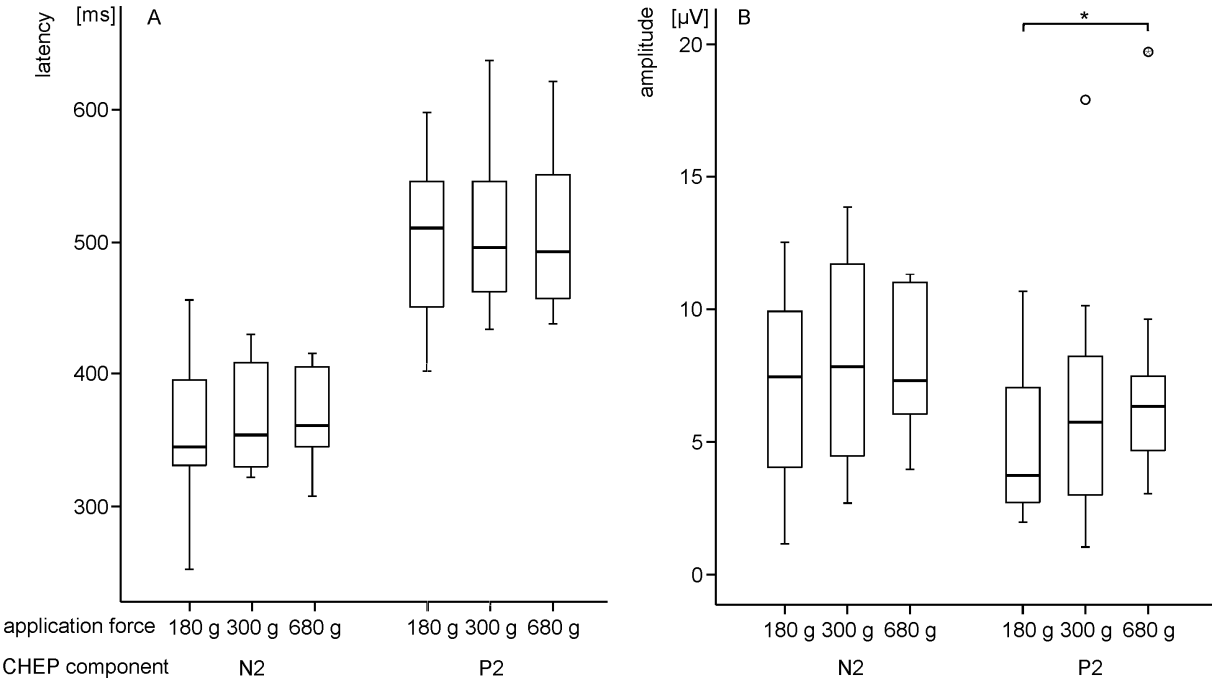

Fig. 3.3: CHEP latency A and amplitude B to stimulation with different thermode application forces (180g, 300g, 680g). Amplitudes of the N2 component were rectified. Boxes indicate values between the 25th and 75th percentile. The horizontal bar reflects the median and the minimum and maximum of whiskers data not statistically outlying (<1.5 interquartile range away from the edges of the box), o = outlier, *=P<0.05, Wilcoxon test

3.4.3 Heat transfer model

A stimulus of 54° C (nominal) penetrated less than 1 mm into the tissue, see Fig. 3.4. For comparison with experimental values (Wydenkeller et al., 2008) the nominal stimulation temperatures fed into the model were defined as threshold temperatures. Thus, latencies from increase of thermode temperature to the peak in tissue temperature linearly increase with higher threshold, see Fig. 3.5. Linear regression

indicates a slope of 15 ms/° C for this linear dependence. The temperature peak in pressure condition gap_skin/air took 16 ms longer to reach the nociceptor level than in the condition gap/skin and only was 42.6° C instead of 43.2° C at nociceptor level.

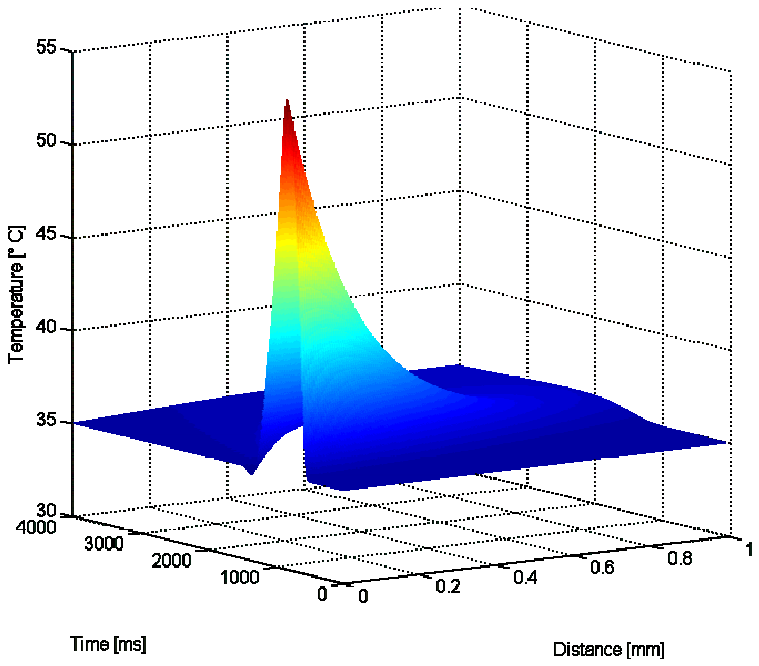

Fig. 3.4: Temperature distribution in the skin after a 54° C stimulus in the first millimetre of tissue. The x-axis represents the distance from the body surface, the y-axis represents the time from start of thermode temperature rise and the z-axis represents the temperature. The heat penetration into the tissue is small and the tissue temperature reaches normal body temperature before less than 1 mm.

3.5 Discussion

Contact heat stimulation at different temperatures above pain threshold modulated the pain perception and the CHEP amplitude. While CHEP latency of the N2 components remained constant, the CHEP was considerably altered in the time window of the positive component. Its topographical variability suggests different processes as generators rather than a P2 component of variable latency. Thus, it could be that with lower temperatures and thus lower stimulation intensity P2 vanished and/or was superimposed by another component. This late positive component could be P3-like related to attention, appearing due to the low stimulation intensity in combination with the long inter-stimulus interval and the rating task, (Becker et al., 2000; Becker et al., 1993; Kanda et al., 1996; Legrain et al., 2002; Mouraux and Plaghki, 2007; Siedenberg and Treede, 1996; Towell and Boyd, 1993). Another explanation for the delayed positive peak with lower stimulation could be

unmasking of C-fiber excitation. These fibers conduct more slowly than Aδ-fibers supposed to be involved in N2 and P2 generation and are already excited by lower stimulation temperature. It is hypothesized, that as long as Aδ-fiber mediated potentials occur, no C-fiber evoked potential can be recorded (Bragard et al., 1996; Opsommer et al., 1999; Qiu et al., 2001). In contrast to the simultaneous appearance of Aδ- and C-fiber mediated evoked potentials in disease (Granot et al., 2001) this has not been confirmed in healthy subjects (Mouraux and Plaghki, 2007). Furthermore, for being evoked by C-fiber excitation, this late component occurs too early when compared to the related painful laser stimulation (Baumgartner et al., 2005; Bragard et al., 1996; Magerl et al., 1999; Opsommer et al., 1999; Qiu et al., 2001; Truini et al., 2007). Due to this uncertain identity of the late positive component use of the earlier and more constant N2 as CHEP read out parameter seems generally recommendable.

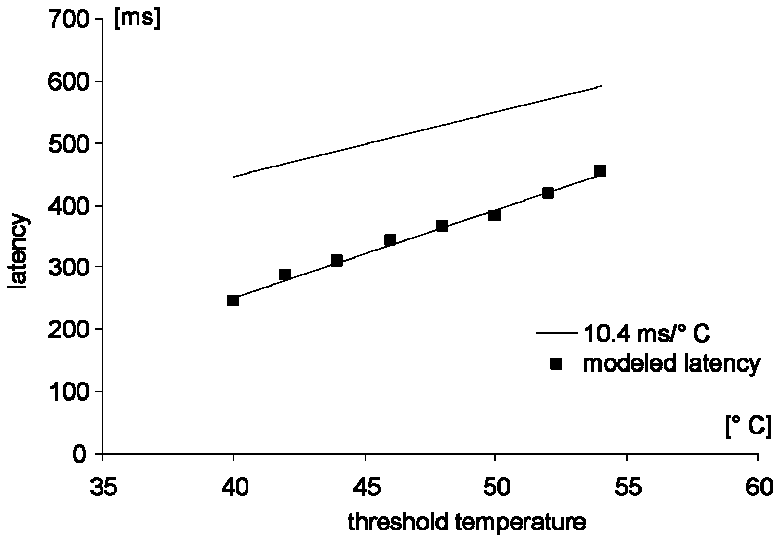

Fig. 3.5: Modelled latencies from rise of thermode temperature to the peak temperature at 0.02 mm nociceptor depth. Latencies are depicted as function of the pain threshold. Inserted the slope of the experimentally determined regression from the pain thresholds vs. P2 contact heat evoked potential (CHEP) latency (Wydenkeller et al., 2008). Note the similarity between the dependence determined in the model and measured in the experiment. The time needed to transfer the heat in the skin (modelled latency), is only a fraction of the total CHEP P2 latency. This explains the difference in intercept between the regression line for modelled and measured latency.

The steep decline of maximal temperature with increasing depth in the skin indicates the considerable influence nociceptor depth could have on CHEP. It could be hypothesized that if nociceptors are not consistently situated at constant depth across different locations or subjects, this could contribute to varying pain thresholds.

Furthermore the close similarity between the measured (Wydenkeller et al., 2008) and computed relationship of pain threshold temperature and latency strengthens the suggestion of the dependence of CHEP latencies on thresholds and validates the model. However, the suggested correction of latencies for pain thresholds must not be applied if thresholds are altered due to the impaired conduction between the nociceptors and the brain, e.g. through a spinal injury.

Variation of the thermode application force neither significantly influenced CHEP component topographies nor the corresponding latencies or heat pain thresholds. Furthermore the N2 amplitude remained unchanged while the P2 amplitude was lower for less force applied. Therefore mainly the P2 amplitude seems to correspond to the pain rating which was also reduced with less applied force. It could be that the P2 amplitude is more strongly than the other parameters influenced by subjective perception. With respect to CHEP latencies and N2 amplitude, the experimental and heat transfer model results do not seem to correspond because they changed only in the model when skin contact was altered. However, the differences between the modeled conditions were rather small so that in the experiment we might not have been able to detect them. Furthermore we took great care to ensure good and even skin contact so the heat transfer might not have improved substantially enough to influence CHEP latencies. Another possibility is that higher pressure might increase the stimulated area and thus excite more nociceptors. This could result in isolated increase of CHEP amplitudes without affection of latencies. Why the influence of thermode application force on pain ratings and pain thresholds is different although both assess the subjective pain perception of individual subjects cannot be answered by this study.

In conclusion, the data presented in this study proved the critical influence of the pain threshold on determination of CHEP latency. On the contrary, the influence of the force with which the thermode is applied may be neglected, at least for investigations on CHEP latency. Mainly the N2 CHEP component should be analyzed in investigations of CHEP latencies and amplitudes in future studies.

4 Study 3: Neuropathic Pain in Spinal Cord Injury: Significance of Clinical and Electrophysiological Measures[3]

4.1 Abstract

A large percentage of spinal cord injured subjects suffer from neuropathic pain below the level of the lesion (bNP). The neural mechanisms underlying this condition are not clear. The aim of this study was to elucidate the general effects of spinal deafferentiation and of bNP on EEG activity. In addition the relationship between the presence of bNP and impaired function of the spinothalamic tract was studied. Measurements were performed in complete and incomplete spinal cord injured subjects with and without bNP as well as in a healthy control group. Clinical examinations revealed a trend towards an association between incomplete lesion and the presence of bNP. Spinothalamic tract function, assessed by contact heat evoked potentials, did not differ between subjects with and without bNP; nevertheless it was impaired in 94 % of subjects suffering from bNP. In the EEG recordings the degree of deafferentiation was reflected in a slowing of EEG peak frequency in the 6-12 Hz band. Taking into account this unspecific effect, spinal cord injured subjects with bNP showed a significantly slower EEG than subjects without bNP. A discrimination analysis in the subjects with spinothalamic tract dysfunction correctly classified 84 % of subjects to either the group with or without bNP according to their EEG peak frequency. This newly identified marker for bNP in spinal cord injury will be helpful both for an objective diagnosis of bNP and for judging the effectiveness of new therapeutic agents.

[3] This manuscript was submitted to the journal: European Journal of Neuroscience; Neuropathic Pain in Spinal Cord Injury: Significance of Clinical and Electrophysiological Measures. The authors were Susanne Wydenkeller, Stefano Maurizio, Volker Dietz and Pascal Halder. The measurements and analyses were conducted by Susanne Wydenkeller. The manuscript was written by Susanne Wydenkeller and revised by the co-authors.

4.2 Introduction

Neuropathic pain (NP) was recently defined as “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system” (Treede et al., 2008) and is therefore not directly associated with nociceptive input. NP can occur in many conditions such as multiple sclerosis, stroke, limb amputation, peripheral nerve lesion or spinal cord injury (SCI). In SCI, NP affects approximately half of all subjects (Siddall et al., 2003; Werhagen et al., 2004) and significantly reduces quality of life (Summers et al., 1991; Anke et al., 1995). It is assumed that in SCI different types of NP are linked to different pathological mechanisms (Siddall et al., 2000; Beric, 2003). NP below the level of the lesion (bNP), which is studied here, is thought to be associated with damage to the central nervous system (Siddall & Loeser, 2001).

Although the underlying neural mechanisms of bNP in SCI are unknown, the involvement of dysfunction of the spinothalamic tract (STT), which mainly mediates pain and temperature sensation, has been assumed (Defrin et al., 2001; Finnerup et al., 2003a; Finnerup et al., 2007). These studies have shown that STT dysfunction is a crucial but not predictive condition for the development of bNP. However, it has not been possible to link the occurrence of bNP to the extent of STT dysfunction, suggesting that additional mechanisms might contribute to the development of bNP after SCI.

Such a view is supported by another line of evidence linking central NP to changes in thalamocortical networks. These changes are associated with slowed EEG, indicative of thalamocortical dysrhythmia (Llinas et al., 1999; Sarnthein et al., 2006). In NP of variable origin a decrease in the EEG peak frequency compared to that of control subjects was detected and thalamectomy resulted in normalization of EEG as well as pain relief (Sarnthein et al., 2006). This suggests a closer link of changes in EEG peak frequency than of STT dysfunction with the occurrence of bNP.

In SCI subjects, independently of the presence of NP, a decrease in the EEG peak frequency occurs (Tran et al., 2004; Herbert et al., 2007; Boord et al., 2008). This suggests that the central deafferentiation per se leads to slowed EEG. Up to now, the effects of deafferentiation and of bNP on EEG peak frequency have not been separated.

Therefore this study aims to disentangle effects of central deafferentiation and bNP on thalamocortical rhythms. In addition, we use the novel method of contact heat evoked potentials (CHEP) to objectively assess STT dysfunction (Chen et al., 2001; Arendt-Nielsen & Chen, 2003; Wydenkeller et al., 2008). This electrophysiological approach has been shown to be specifically related to impulse transmission through Aδ-fibers of the STT (Wydenkeller et al., 2008). It has not yet been systematically used to objectively assess STT dysfunction after SCI. It is hypothesized that by combining the analysis of EEG peak frequency with CHEP an objective discrimination between SCI subjects with and without bNP can be achieved.

4.3 Methods

4.3.1 Subjects

Twenty-six consecutive chronic SCI subjects of the University Hospital Balgrist, Switzerland (age: 47.0 ± 15 years; height 1.74 ± 0.08 m; weight 71 ± 13 kg; duration of injury: 1-47 years; six women; mean ± s.d.) and 26 age- and gender matched control subjects participated in the study. SCI subjects had complete or incomplete lesions at neurological levels C5-T10. Subjects with additional spinal column lesions below T10 were not included. No neurological history except the SCI and mild depression was permitted. Subjects with at level NP were excluded from the study to avoid peripheral mechanisms as pain generators. None of the subjects reported above level NP. For ethical reasons, medication was not tapered off for the examination. See Tab. 4.1 for demographic and medication data. The experiment was approved by the local ethics committee and was conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent.

Subjects were interrogated about their pain sensations and were asked to draw the pain distribution on a body chart. The mean pain intensity in the last two weeks before the measurement was assessed on a numeric rating scale (NRS 0-10, 0 no pain, 10 most intense pain imaginable). With the help of their medical history and a structured interview, the subjects were then classified into two groups according to whether they experienced bNP or not (Siddall et al., 1997). Subjects suffering from other chronic pain sensations (musculoskeletal, visceral) were not included in the group without bNP. Of the 26 SCI subjects, 17 had and 9 did not have bNP. Contact heat stimulation was not conducted in three of the SCI subjects and one SCI subject

did not show alpha activity characteristic of resting EEG and was thus not included in the analysis of EEG peak frequency. The three subjects who could not be included in the analysis of CHEP all had bNP, such that in the CHEP comparisons 14 subjects with vs. 9 without bNP are included. The one subject not included in the EEG peak frequency analysis had bNP, so that 16 subjects with vs. 9 without bNP were compared in these analyses.

4.3.2 Contact heat stimulation for evoked potentials

The skin on the back was stimulated with short heat pulses produced by a contact heat stimulator (Medoc, Ramat Yishai, Israel) described elsewhere (Wydenkeller et al., 2008). Two locations, 6 cm above and below the neurological level of the SCI on each side of the back were stimulated. They were located 5-10 cm away from the midline. Distances between the brain and the stimulated locations in each control subject were adapted to the corresponding gender- and age matched SCI subject. In subjects with complete SCI the location below the SCI was often situated in the border zone of the injury with some preserved function. Subjects sat comfortably in their wheelchair or on a chair without leaning against the backrest. Hyperexcitability at the lesion level was investigated in terms of pinprick sensation and heat pain thresholds. Furthermore, heat pain thresholds were determined for every stimulated location before the main experiment. Subsequently, stimulation temperature was set 3° C higher. Maximal temperature never exceeded 55° C. Two blocks of stimulation were applied during the main part of the experiment. In each block the locations on one body side were alternately stimulated 30 times. The first stimulated side and location was balanced across subjects. The inter-stimulus interval was 40-48 s for the single locations. Placement of the 5.7 cm^2 thermode was random within an area of approximately 15 cm^2. Prompted by an acoustic signal 4 s after stimulation, subjects had to rate the stimulus intensity on a numeric scale from 0 to 10, where 0 meant no pain and 10 the worst imaginable pain. Subjects were instructed to look straight ahead, fixate a point on the wall and not to blink more than necessary.

EEG measurement and analysis

Thirty Ag/AgCl scalp electrodes and two electrodes below the outer canthi of the eyes were used to record the continuous EEG at 500 Hz (QuickAmp, Brainproducts, Munich, Germany). Subject ground was at Afz position. Impedances were kept below

20 kΩ (Ferree et al., 2001). The EEG was analyzed with Brainvision Analyzer software (Brainproducts, Munich, Germany).

4.3.3 Evoked potentials

Raw data was filtered offline from 0.5-30 Hz. Removal of eye blinks and stimulus-locked artifacts (Iannetti et al., 2006) was achieved with an independent component analysis (Jung et al., 2000; Halder et al., 2007). Data was segmented from -100 ms to 2000 ms according to stimulus onset. Trials with artifacts exceeding ± 80 µV were automatically excluded from all analyses. Latency of the CHEP N2 (the first prominent negativity (Chen et al., 2001)) was defined in a time window of 200-650 ms on the Cz electrode against average reference. The latencies were subsequently normalized for the variable lesion levels and thus for variable distances to the brain using an estimated STT conduction velocity of 11.2 m/s (Wydenkeller et al., 2008). Data after stimulation at the upper location on both body sides in the control subjects was used to normalize for known age effects (Dustman et al., 1993) (linear regression, n=52; latency slope=0.966, Spearman ρ=0.423, P=0.002; amplitude slope=0.174, Spearman ρ=0.486, P<0.001,). The STT was defined to be dysfunctional if either no CHEP to stimulation below the SCI could be recorded or if latencies were pathological (exceeding mean+1 s.d. of the control group).

4.3.4 Resting EEG

Prior to the evoked potential recording session, subjects were instructed to close their eyes for the 2-3 min resting EEG recording. Data was cut into segments of 2048 ms and filtered offline from 0.5-30 Hz. Segments contaminated by artifacts exceeding ± 50 µV (except in occipital channels) were discarded. EEG with less than 24 remaining segments (n=10) were subjected to an independent component analysis that removed eye artifacts (Jung et al., 2000). On average 117 s of resting EEG per person were subjected to a fast fourier transformation (10% overlapping Hanning window, resolution 0.488 Hz). The EEG peak frequency was determined as the dominant peak in the average of all channels in a frequency window of 6-12 Hz. In an enlarged group of healthy subjects the EEG peak frequency correlated with age (age range 18-69 years, n=45, Spearman ρ=-0.288, P=0.055, slope of linear regression=-0.016). This dependence has been described before in the literature (Dustman et al., 1993; Klimesch, 1999). Thus, all EEG peak frequencies were normalized to age 42

years to reduce variation in the data using the formula: normalized frequency=frequency-((age-42)*-0.016). Conversely, the logarithmized EEG peak power did not correlate to age in the control group.

4.3.5 Statistics

Frequency of the different grades of SCI, according to the American Spinal Injury Association (Marino et al., 2003), in the groups with and without bNP was analyzed with the 2-sided Pearson's X^2-test. Frequency of hyperalgesia evoked by pinprick and the number of measurable CHEP in the groups to be compared were analyzed by the 2-sided Fisher's exact test. Data was checked for normal distribution using the Shapiro-Wilk's test. CHEP N2 latencies were not normally distributed and were therefore log-transformed for later repeated measures analysis of variance (ANOVA). ANOVA of the N2 latencies with the factors left and right body side, stimulated locations above and below the SCI and groups SCI vs. control subjects was calculated (n=23 control, 15 SCI subjects). The non-parametric 2-tailed Mann-Whitney U test was used for post-hoc tests and for comparing N2 latencies between subjects with and without bNP because latencies of these two groups were not normally distributed even after log-transformation.

Linear regression was used for the analysis of the relationship between the measured parameters and age (EEG peak frequency, logarithmized EEG peak power, CHEP latencies and amplitudes). Furthermore regression between the EEG peak frequency normalized for age and the extent of deafferentiation caused by the SCI as well as the pain intensity was calculated. If data was not normally distributed the significance of the correlation was reassured by non-parametric Spearman correlation. In SCI subjects with complete absence of pain sensation to a 55° C contact heat pulse below the zone of partial preservation, the number of segments below the sensory lesion level was defined as the extent of deafferentiation. In the remaining subjects the number of segments below the SCI was divided by 2 to account for the only partial lesion. Differences in EEG peak frequency and power between SCI and control subjects (n=22 SCI and 45 control subjects) or SCI subjects with and without bNP were tested with non-parametric 2-tailed Mann-Whitney U tests. Due to the potential influence of neuroactive medication on EEG peak frequency the analyses on this parameter were also conducted in a subgroup of subjects free of medication. Discrimination analysis with cross-correlation was used

to classify the SCI subjects with STT dysfunction to either the group with or without bNP according to their EEG peak frequency. As STT dysfunction was regarded as precondition for bNP, only subjects with dysfunction were included in this analysis. Level of significance for all tests was defined as $P<0.05$. All statistical analyses were performed in SPSS 14.0.2. (SPSS Inc., Chicago, USA).

4.4 Results

4.4.1 bNP and severity of SCI

Characteristics of SCI subjects are presented in Tab. 4.1. bNP was symmetrically distributed on both sides of the affected subjects, except in one who felt more bNP on the left side. A trend towards higher prevalence of bNP in subjects with a less severe lesion according to the clinical ASIA impairment scale (AIS) was detected, as all subjects with AIS grade C or D suffered from bNP (X_3^2=6.6 (n=26), P=0.087). Hyperalgesia to pinprick stimulation at the lesion level was equally present in subjects with and without bNP (X_1^2=1.1 (n=26), P=0.380). Neither were heat pain thresholds different at lesion level between these subjects on the left or right side (left z=-0.49, P=0.627 and right z=-0.37, P=0.713).

subject	gender	age	level of SCI	AIS grade	neuroactive medication	years post injury	lesion type	bNP	pain intensity
1	F	44	T8	A	AI	12	T	yes	7
2	M	68	T10	A	-	40	T		
3	M	56	C7	A	-	15	T		
4	F	32	T5	A	-	14	T	yes	5
5	M	22	T4	A	-	3	T		
6	F	49	T6	A	-	17	T		
7	M	36	T3	A	-	15	T	yes	3
8	M	29	T4	A	-	2	G	yes	5
9	M	41	T5	A	-	19	T		
10[b]	M	45	T4	A	AC	1	T	yes	8
11	M	40	T2	A	AE	1	T	yes	5
12	M	28	T6	A	AE, S	4	T	yes	3
13[a]	F	26	T4	A	-	6	G	yes	3
14	M	67	C8	A	-	47	T		
15	F	54	T2	A	AD	29	T		
16	M	48	T4	B	-	3	T	yes	4
17	M	18	C6	B	-	1	T		
18	M	38	T5	B	-	18	T		
19	M	67	C5	C	-	6	T	yes	6
20	M	49	C7	D	-	3	T	yes	6
21[a]	M	63	T6	D	AD	6	T	yes	5
22	M	68	C5	D	AE, O, S	3	T	yes	4
23	F	60	C6	D	AE, S, AC	1	T	yes	0[c]
24	M	60	C6	D	-	5	T	yes	2
25[a]	F	48	C6	D	-	3	T	yes	4
26	M	65	C6	D	AI	6	T	yes	5

Tab. 4.1: Spinal cord injury (SCI) subject characteristics. AIS grade: severity of lesion according to American Spinal Cord Injury Association (Marino et al., 2003). bNP = below level neuropathic pain; AE = antiepileptics, S = spasmolytics, AD = antidepressiva, O = opioids, AI = non-steroidal anti-inflammatory drugs, AC = anticholinergics, T = traumatic, G = gunshot wound, [a] = not included in the analysis of contact heat evoked potentials; [b]=not included in the analysis of EEG peak frequency; [c]=not experiencing bNP due to successful medication.

4.4.2 Contact heat evoked potentials

In the control subjects, CHEP were consistently recorded to stimulation at all locations, while in SCI subjects this was only the case when stimulation was applied above the SCI (percentage of absent CHEP to stimulation below SCI for sides: right 30%, left 13%). For stimulation below the lesion, differences between SCI and control subjects manifested as reduced amplitude and delayed latency of the N2 component in SCI subjects (see Fig. 4.1A and Fig. 4.1B).

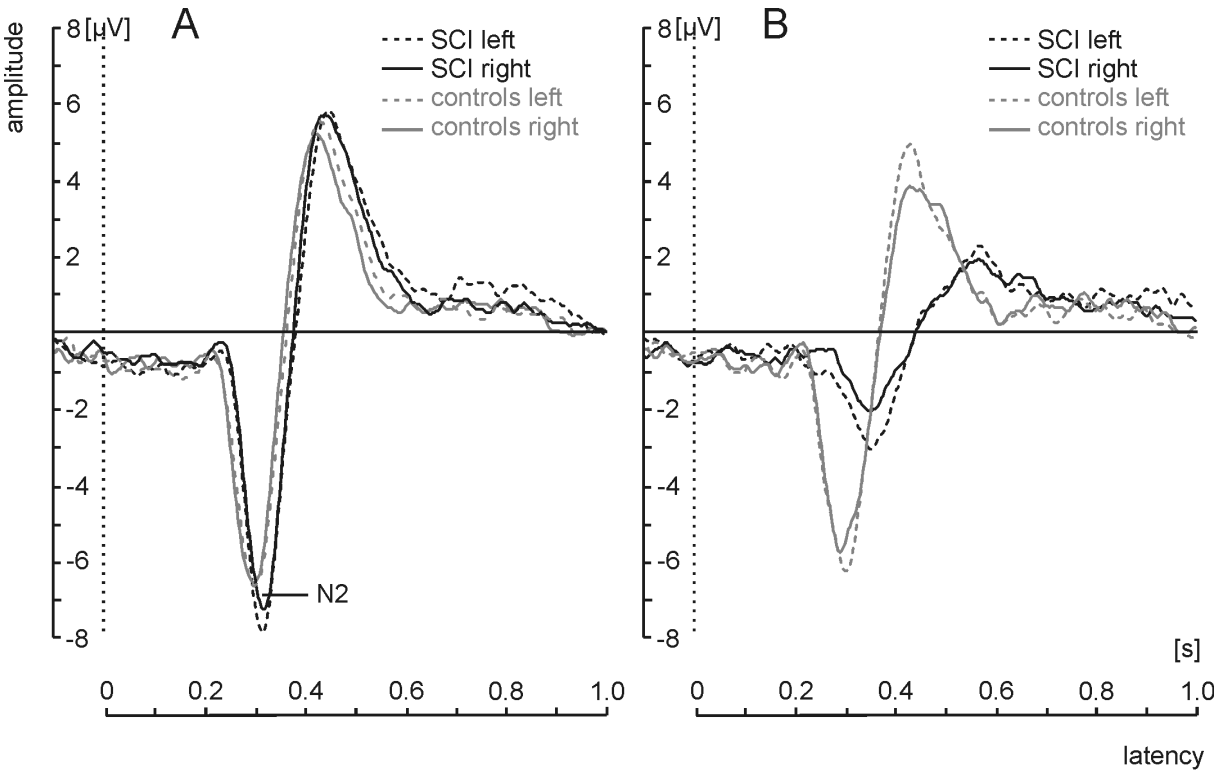

Fig. 4.1: Contact heat evoked potentials. Shown on the Cz vertex electrode against average reference, after stimulation on the left and right side **A** above and **B** below the lesion in spinal cord injured (SCI) subjects and in the control group. The N2 latency was significantly longer in the SCI compared to the control subjects to stimulation below the lesion on both (P<0.001) and above the lesion on the right side (P=0.001).

Longer N2 latencies to stimulation below the lesion in SCI subjects were present according to ANOVA (main effect of location with longer latencies to stimulation below than above the lesion, $F_{1,35}$=34.0, P=0.000; significant interaction group*location, F=21.8, P<0.001). The interaction group*side (F=4.3, P=0.047) was due to longer latencies to stimulation above the lesion on the right but not on the left side in the SCI subjects (post hoc Mann-Whitney U test, left z=-1.23, P=0.220, right

z=-3.28, P=0.001). CHEP N2 amplitudes to stimulation below the lesion were smaller in SCI subjects (main effect of location with smaller amplitudes below than above the lesion, $F_{1,35}$=9.6, P=0.004; significant interaction group*location, F=14.2, P=0.001). No interaction group*side (F=0.253, P=0.618) was detected for the amplitudes.

In 94 % of the SCI subjects suffering from bNP a STT dysfunction, as defined by missing CHEP or prolonged latency when stimulation was applied below the level of lesion, was diagnosed. Neither presence nor absence of CHEP nor latencies or amplitudes of the CHEP N2 allowed for differentiation between subjects with and without bNP, see Tab. 4.2.

	stimulation above the SCI			
	latency [ms]		amplitude [µV]	
	median/range/n		median/range/n	
	left	right	left	right
without bNP	317/281-347/9	341/278-390/9	9.7/3-20/9	9.8/5-19/9
with bNP	320/276-366/14	330/274-432/14	7.4/4-18/14	6.9/2-19/14
z, P[a]	-0.32, 0.75	-0.32, 0.75	-0.19, 0.85	-1.64, 0.10
	stimulation below the SCI			
	left	right	left	right
without bNP	359/257-628/9	393/300-611/8	5.6/1-13/9	5.6/1-12/8
with bNP	388/304-487/11	397/306-466/8	5.2/1-12/11	5.4/1-8/8
z, P[a]	-0.95, 0.34	-0.11, 0.91	-0.34, 0.73	-0.32, 0.75

Tab. 4.2: Latencies and amplitudes of the contact heat evoked potential N2 component. Amplitudes were rectified. bNP: below level neuropathic pain. [a] *z* and *P* values of the Mann-Whitney U tests.

4.4.3 EEG peak frequency

The EEG peak frequency was significantly slower in SCI than in control subjects (z=-2.10, P=0.038), see Fig. 4.2A. The central deafferentiation through the SCI was associated with a decreased EEG peak frequency. In the subjects without bNP and without pain sensation below the zone of partial preservation, the EEG peak frequency showed a significant relation to the extent of deafferentiation with a

slowing of peak frequencies corresponding to more injured segments (n=8, R^2=0.782, P=0.004, slope of linear regression=-0.219), see Fig. 4.2B.

In a second step, the EEG peak frequency was normalized for the number of injured segments, using the slope of the regression from Fig. 4.2B in the formula: normalized frequency=frequency-((injured_segments-19)*-0.219. In the subjects without pain sensation below the zone of partial preservation the number of segments below the SCI was divided by 2 to account for the only partial lesion. No separate normalization slope was calculated for this group as all subjects suffered from bNP. This normalized EEG peak frequency was slower in the SCI subjects with than in those without bNP (z=-2.15, P=0.031), see Fig. 4.2C. Exclusion of subjects taking neuroactive medication from the EEG peak frequency analysis did not change this difference (n=8 without, n=9 with bNP, z=-2.12, P=0.034). The EEG peak frequency of subjects with bNP did not correlate to the mean pain intensity experienced in the two weeks before the measurement, whether subjects on medication were included or not.

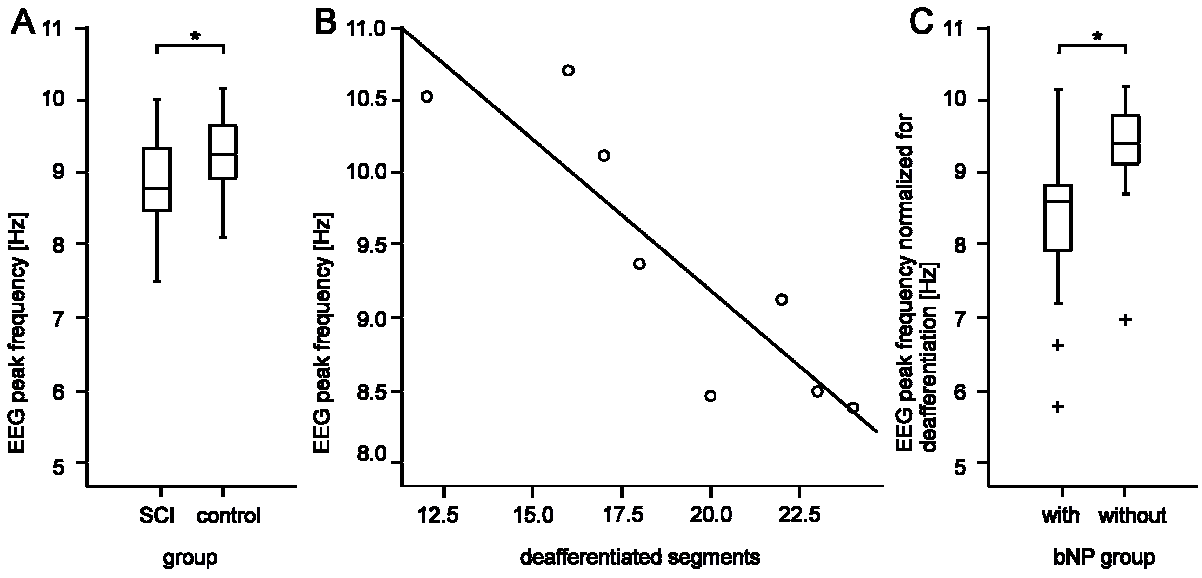

Fig. 4.2: EEG peak frequency. **A** The EEG peak frequency of the spinal cord injured (SCI) subjects is significantly lower than that of the control subjects (P=0.038). **B** In subjects with complete SCI but without below level neuropathic pain (bNP) the EEG peak frequency correlates significantly with the extent of deafferentiation as defined by the number of injured segments. C The EEG peak frequency normalized for deafferentiation was significantly lower in the SCI subjects with than in those without bNP. Boxes indicate EEG peak frequencies between the 25th and 75th percentile. The horizontal bar reflects the median. Minimum and maximum of whiskers indicate data not statistically outlying (<1.5 interquartile range away from the edges of the box), + = outlier, *=P<0.05, Mann-Whitney U test.

Subsequently, subjects with STT dysfunction were classified to either the group with or that without bNP. With this discrimination analysis using the normalized EEG peak frequency, 84.2% of all SCI subjects could be classified to the correct group (n=19, λ=0.738, P=0.025). In contrast to the EEG peak frequency, the peak power did not correlate with either extent of deafferentiation or bNP.

4.5 Discussion

The aim of this study was to identify clinical and electrophysiological markers for the occurrence of bNP in SCI subjects and to determine their diagnostic usefulness. In line with others (Defrin et al., 2001; Finnerup et al., 2003a; Finnerup et al., 2007) we found STT dysfunction by recording CHEP in the large majority of SCI subjects with bNP. Conversely, STT dysfunction was not different between subjects with and without bNP. However, through the use of the EEG peak frequency SCI subjects suffering from bNP could be separated from those without bNP. This effect could only be unmasked by considering unspecific effects of deafferentiation.

4.5.1 bNP and clinical deficit

In this study, subjects with incomplete SCI (AIS grade B, C or D) suffered more frequently from bNP than those with a complete SCI (AIS grade A). The relationship between the completeness of a SCI and the occurrence of NP is controversially discussed (Beric et al., 1988; Davidoff et al., 1987; Finnerup et al., 2003b; Siddall et al., 2003; Siddall et al., 1999; Werhagen et al., 2004). Nevertheless, a recent report also linked the occurrence of NP to ‘discomplete’ STT lesions (Wasner et al., 2008). In earlier studies using magnetic resonance images or quantitative sensory testing, no clear differences between the groups with or without bNP emerged (Defrin et al., 2001; Eide et al., 1996; Finnerup et al., 2003a; Finnerup et al., 2003b; Finnerup et al., 2007). The main finding of these studies was abnormal evoked pain, either at or below the SCI in subjects with bNP. Here, this result could not be confirmed, probably due to the strict exclusion of subjects with at level NP.

4.5.2 bNP and spinothalamic tract dysfunction

For the first time we have been able to show that CHEP recordings allow objective quantification of the impaired function of Aδ-fibers in the STT after a SCI, whereas only single cases were presented before (Wydenkeller et al., 2008). While clinical

examinations suggested that partial STT lesions seem to be associated with bNP after SCI, this was not reflected in the CHEP recordings. Unexpectedly, the CHEP latencies to stimulation above the lesion were also minimally delayed. With regard to normal light touch and pinprick sensation at the stimulated location, this finding implies that CHEP are sensitive enough to detect subclinical deficits. Similarly, discrepancies between clinical and perception threshold examinations were described earlier (Hayes et al., 2002; Savic et al., 2006; Kramer et al., 2008). Here we confirm that impaired STT function is a precondition, although it is not predictive, for the development of bNP. The one subject suffering from bNP and with normal CHEP had a minimal SCI with slight sensory/motor loss and bNP was only present in the one arm in the T1 segment. In this subject it can not be excluded that this pain was rather at level NP than bNP as it was located just three segments below the SCI.

4.5.3 bNP and thalamocortical dysrhythmia

EEG peak frequency showed a clear difference between SCI subjects with and without bNP. Primarily this effect was masked by a slowing of the EEG due to the central deafferentiation. Furthermore, this slowing was correlated to the extent of deafferentiation, as determined in subjects with complete absence of pain sensation below the zone of partial preservation and not suffering from bNP. However, after normalization for deafferentiation the EEG peak frequency was slower in the subjects with than in those without bNP.

Slowed EEG caused by deafferentiation is explained by the concept of thalamocortical dysrhythmia (Llinas et al., 1999; Llinas & Steriade, 2006). Thalamocortical dysrhythmia is thought to be initiated by sensory deafferentiation of thalamic cells ultimately leading to cell hyperpolarisation and firing at slower frequencies. The fact that this EEG slowing was more pronounced in bNP is a finding whose underlying mechanism can hardly be explained by this study. While EEG slowing in SCI subjects was mentioned earlier (Tran et al., 2004; Herbert et al., 2007; Boord et al., 2008), the quantification of the differential influence of deafferentiation and of bNP is novel. Deafferentiation after SCI was shown to have an effect on metabolic activity in thalamic nuclei using MR spectroscopy (Pattany et al., 2002). Correspondingly, in an experimental SCI rat model, the firing pattern in thalamic cells was found to be altered. In addition to this general change, further abnormal activity was specific to rats with NP (Gerke et al., 2003).

Neuroactive medication might influence EEG peak frequency, which was however not the case in this study. Thus the slowed EEG peak frequency in subjects suffering from bNP was unlikely caused by neuroactive drugs. It is noticeable that less than half of the SCI subjects suffering from bNP took strong analgesic medication despite their ongoing pain. This can be explained by the fact that bNP is not easily treatable by drugs (Siddall et al., 2000).

The present findings allow discrimination between subjects with and without bNP. However, it remains unclear what ultimately leads to bNP after damage to the STT, whose dysfunction might trigger a cascade of plastic changes. This suggestion is supported by the rather late onset of bNP after injury (Siddall et al., 2003) and the development of allodynia induced by a regenerative treatment in SCI rats. Various other factors like personal experience and mental state or coping strategies should also be taken into account as these are known to influence the occurrence of bNP (Summers et al., 1991; Widerstrom-Noga, 2003; Widerstrom-Noga et al., 2007).

In conclusion, this is the first study to show a specific association of bNP with a slowing of EEG peak frequency. In combination with the novel electrophysiological method for quantifying STT dysfunction, SCI subjects with and without NP can be discriminated objectively. This will be of importance in assisting diagnosis and judging the effectiveness of new therapeutic agents.

5 Study 4: Association between Enhanced Recovery of Spinothalamic Function and Below Level Neuropathic Pain[4]

5.1 Abstract

Spinothalamic tract (STT) dysfunction seems to be crucially involved in the development of central neuropathic pain (NP) after spinal cord injury (SCI). However, previous attempts to identify differences in extent or location of STT damage between subjects with and without NP failed. Here we show that the spontaneous recovery of human STT function (within the first year after SCI) in subjects suffering NP is enhanced compared to those not affected. Furthermore, the correlation between current pain intensity (assessed on average 5 years after SCI) and extent of functional recovery substantiates the close relationship between recovery of STT function and the occurrence of NP. These findings contribute to a better understanding of mechanisms involved in the generation of NP after SCI.

5.2 Manuscript

Despite the high clinical relevance of central neuropathic pain (NP) after spinal cord injury (SCI) the underlying mechanisms are still unclear. Functional impairment of spinothalamic tract (STT) pathways, mainly conducting temperature and pain sensation from the periphery to the brain seem to be crucially involved (Defrin et al., 2001; Eide et al., 1996; Finnerup et al., 2003b). These impairments, however, are not predictive. More specifically, no differences in the extent or location of STT damage could be identified between subjects with and without NP (Defrin et al., 2001; Finnerup et al., 2007). This strongly suggests additional mechanisms, probably induced by the STT lesion, to be involved in the pathogenesis of NP. Several lines of

[4] This manuscript is going to be published as brief communication in Experimental Neurology: Enhanced recovery of human spinothalamic function is associated with central neuropathic pain after SCI; Experimental Neurology, 2009, in press. The authors are Annegret R. Hari, Susanne Wydenkeller and Pascal Halder. Data was assessed by Annegret R. Hari and the EM-SCI-study group. The analyses were conducted by Annegret R. Hari. The manuscript was written by Annegret R. Hari and revised by the co-authors.

evidence suggest aberrant spinal plasticity as an important factor in NP generation. First, both animal and human studies have shown that sprouting of primary afferent nerve fibers occurs spontaneously after SCI and might be associated with NP states (Ackery et al., 2007; Bennett et al., 2000; Christensen and Hulsebosch, 1997). In addition, aberrant axonal sprouting induced by stem cell grafts is linked to NP behavior in rodents (Hofstetter et al., 2005). Finally, the rather late onset of NP after human SCI (weeks up to several years) seems in accordance with an involvement of plastic changes. We thus tested the hypothesis that enhanced spontaneous recovery of STT function is associated with the occurrence of NP. For testing specificity dorsal column function and recovery were also assessed.

Subject	Sex	Age	Age at injury	Years between injury and pain interview	Level	ASIA grade	Neuroactive medication	Lesion	bNP
1	M	68	61	6.1	C5	C	-	T	x
2	M	44	37	7.0	T12	A	AD	T	x
3	F	45	38	7.0	C3	D	AE, O	I	x
4	M	61	55	5.0	C2	D	-	T	x
5	M	52	46	6.0	T8	A	AE	T	x
6	F	21	15	5.9	T8	B	AE	I	x
7	M	30	27	1.9	T4	A	S, AC	T	x
8	M	47	45	2.0	C7	B	AE	T	x
9	M	33	26	7.0	C5	C	-	T	
10	M	36	30	6.3	C4	C	-	T	
11	M	54	48	5.8	T10	A	AI	T	
12	M	42	37	5.1	T12	B	-	T	
13	F	28	23	5.1	C6	D	AI	T	
14	F	49	44	4.9	T11	C	-	T	
15	M	23	19	3.1	C7	A	-	T	
16	M	79	75	4.1	C5	D	-	T	

Tab. 5.1: SCI subject characteristics. AE = antiepileptics, S = spasmolytics, AD = antidepressiva, O = opioids, AI = non-steroidal anti-inflammatory drugs, AC = anticholinergics, T = traumatic, I = ischemic,bNP = below level neuropathic pain

In total 28 SCI subjects from the University Hospital Balgrist, Switzerland, were examined twice within the first year after injury (first examination 13±9 days post injury, second examination 324±57 days post injury, mean±SD). None of them had a

psychiatric or neurological disease, except for the SCI. For subjects characteristics, including data on medication, see Tab. 5.1. All subjects gave written informed consent and the study was conducted in accordance with the guidelines of the Declaration of Helsinki. Pinprick and light touch sensations were assessed in every segment on a three point scale (0= absent, 1= impaired, 2= normal sensation) following the “International Standards for Neurological Classification of Spinal Cord Injury” guidelines (Marino et al., 2003). Pinprick sensation is considered to be indicative of STT function whereas light touch is associated with dorsal column function. These parameters have been shown to improve within the first year after injury (Fawcett et al., 2007; Spiess et al., 2008). In order to investigate the mechanisms underlying NP, we compared both the recovery of pinprick and light touch scores within the first year after SCI between the groups with and without central NP. These groups were defined after classification of at-level and below-level NP (bNP) according to a structured interview and a standardized classification system (Siddall et al., 1997). Pain interviews were conducted 5.13±1.63 years (mean±SD) after injury, but not earlier than 1.8 years after injury, which corresponds to the reported mean onset time of bNP (Siddall et al., 2003). Inclusion criteria for the group without bNP were no intermittent or continuous NP at or below the level of injury. In the group with bNP, subjects with bNP only were included in data analyses, as at-level NP is thought to involve different mechanisms including peripheral nerve damage (Siddall, et al., 1997). The groups (eight with bNP, eight without) did not statistically differ in age, age at injury, lesion level, completeness of lesion and in the time span between injury and the assessment stages. In addition, no systematic difference in medication between the groups is apparent, making an important contribution of medication on the outcome of the study unlikely. As a part of the pain interview subjects were also asked to rate both the current (at the moment of the questionnaire) and maximal pain intensity on a numerical rating scale (0= no pain, 10= worst imaginable pain) (Bryce, et al., 2007). Two subjects within the pain group rated the current pain intensity as 0 (one subject with intermittent pain and one with successful treatment by antiepileptic medication). For statistical analyses the mean of the pinprick or light touch scores of five segments of both body sides below the last dermatome with normal sensory function was used (these dermatomes were not always identical for the body sides and the modalities). We decided to focus our analyses on these segments as they usually exhibit preserved sensory function and

thus enhanced recovery potential also in subjects with complete lesions (zone of partial preservation). In four subjects (two with and two without bNP), pinprick scores decreased over time (the mean decrease was 0.275). These four patients were excluded from the analyses of pinprick scores as they did not show the expected pattern of sensory recovery. As we a priori hypothesized that larger STT recovery is associated with the occurrence of bNP, these subjects were excluded from the pinprick score analysis. Consequently, three patients with loss of dorsal column function were excluded from the corresponding analysis of the recovery of light touch scores.

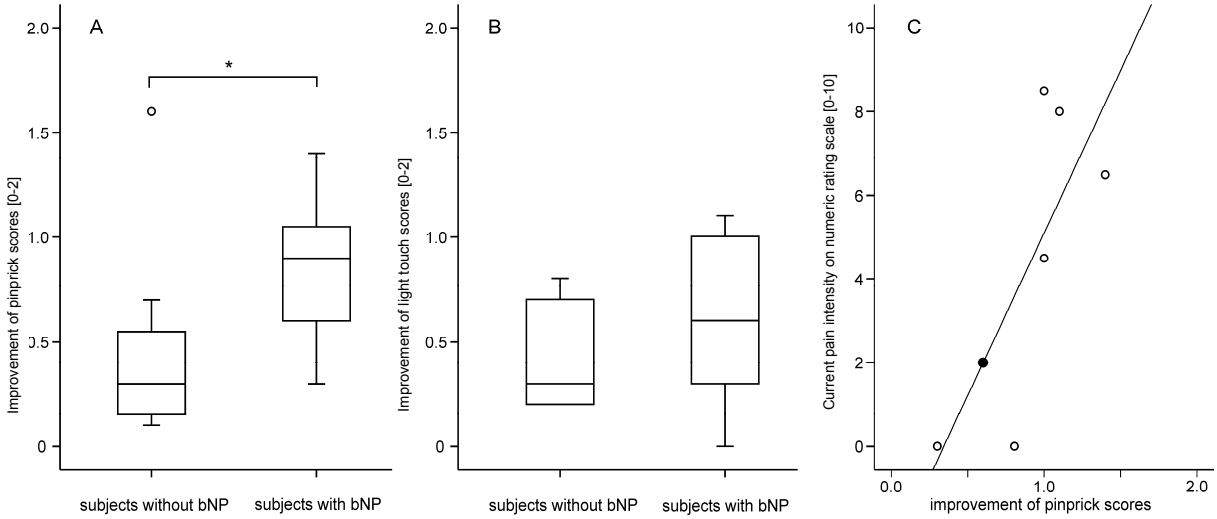

Fig. 5.1 Improvement of **A** pinprick and **B** light touch scores within the first year after injury in subjects with and without below level neuropathic pain (bNP). The scores are the mean of 5 segments of both body sides below the last dermatome with normal sensory function. Boxes indicate the 25th and 75th percentile. The horizontal bar represents the median. Minimum and maximum of whiskers indicate data not statistically outlying (<1.5 interquartile range away from the edges of the box). **C** Current pain intensity of below-level neuropathic pain correlates with the improvement of the pinprick scores within the first year after injury (r= 0.783, P= 0.022). Each subject is represented by an open circle; a filled circle indicates two subjects.

Improvement of the pinprick scores is significantly larger in the subjects with bNP compared to those without (Mann-Whitney U test (MWU) P= 0.045, see Fig. 5.1A). The scores of neither the early nor the later examination were significantly different, although a tendency for larger STT dysfunction at the early examination was observed in the subjects with bNP (MWU: early examination P=0.091, later examination P=0.958). Altogether, these findings show that the recovery of STT function and not the dysfunction per se is important in differentiating subjects with

and without bNP. Additionally, within the subjects suffering bNP the extent of improvement of the pinprick scores within the first year after SCI correlated positively with the current pain intensity (Spearman's r= 0.783, P= 0.022, see Fig. 5.1C) but not with the maximal pain intensity. In contrast, no differences between subjects with and without bNP could be detected in recovery of the light touch scores (MWU: P= 0.472, see Fig. 5.1B). Improvement of pinprick and light touch scores were not correlated (Spearman's r=0.197, P=0.465).

STT dysfunction has repeatedly been associated with bNP but it remained unclear why only a proportion of subjects exhibiting STT damage develop bNP (Defrin, et al., 2001; Finnerup, et al., 2007). While these studies assessed STT damage and dysfunction at a given time point after injury our study assesses both initial, trauma induced STT dysfunction and its subsequent recovery. Interestingly, in accordance with the aforementioned studies, neither at the earlier nor at the later examination a statistically significant difference in STT function could be assessed. However, differences between SCI subjects with and without bNP were detected in the extent of STT recovery. We show that subjects who develop bNP exhibit enhanced recovery in STT function during the first year after SCI compared to those not suffering bNP. This pattern was not detected in dorsal column function, suggesting a specific involvement of STT recovery. Furthermore, the correlation between current pain intensity, although a strongly subjective parameter, and improvement of STT function further substantiates the suggestion of a causal relationship. However, the current pain intensity could also be influenced by various other factors, such as testing environment or individual mood.

Certainly, the simple neurological assessment on a three point scale as performed in this study is not suitable to directly monitor the complex neuronal mechanisms presumably involved in bNP generation. Consequently, this study leaves open whether the presented findings are causally involved in bNP generation or rather represent an epiphenomenon. It has been suggested that plastic changes just around the lesion level are involved in the generation of bNP (Finnerup, et al., 2007) and our findings might be a direct consequence of these plastic changes. Alternatively, as we measured recovery remote from the location of bNP, it could be hypothesized that our results reflect the overall potential of an individual for the recovery of STT function and as a consequence the mechanisms ultimately leading

to bNP might occur at other levels of the neuraxis. It is also noteworthy that a small proportion of the patients showed a decline in STT function instead of the expected recovery pattern and half of those developed bNP. Thus, functional STT recovery, as assessed in this study, does not seem mandatory for the development of bNP. However, due to the limited amount of subjects exhibiting a functional decline, no firm conclusions on the relation between worsening of STT function and the occurrence of bNP can be drawn.

Whatever the exact mechanism might be it is important to note that new therapies which intend to promote sensory and motor recovery after SCI could simultaneously induce NP by boosting recovery of STT function. Alarming reports warning of these consequences have been published (Deumens, et al., 2008) and our results strongly support these findings.

In conclusion, this study shows that enhanced recovery of STT function is associated with the development of bNP after SCI. These findings substantially further the understanding of why some SCI subjects with STT dysfunction develop bNP and some do not.

6 General discussion and conclusions

6.1 Clinical CHEP protocol

Recording of CHEP as presented in chapter 2 confirmed their applicability to objectively measure STT function. Furthermore, due to the correlation of CHEP latency with pain threshold, the importance of controlling for the threshold was postulated. Intensified investigation of this effect in the study of chapter 3 reaffirmed it and ruled out the possibility that the stimulation temperature per se influenced CHEP latency. With regard to future use of CHEP, additional influencing factors were investigated. Thus, latency of the N2 component might be the most reliable measure of STT function. Furthermore, stimulation temperature should be scaled in relation to and should be higher than the pain threshold. Variation of the thermode application force can be neglected as it only modulated pain perception, while CHEP amplitudes were just minimally affected and latencies remained unchanged.

The theoretical modeling of temperature course in the skin to contact heat stimulation confirmed the crucial influence of the pain threshold on latencies. Underlying these influences on CHEP are the inherent properties of contact heat stimulation: Slow heating rate of the thermode and stimulation remote from the nociceptors. For that reason is the delay from start of temperature rise on the skin to nociceptor firing variable and difficult to control. Application of the proposed protocol, however, minimizes these confounders. Another way of avoiding some of the problems with heat transfer when investigating STT function might be to use laser instead of contact heat stimulation as lasers stimulate closer to or even at the location of the nociceptors (Iannetti et al., 2006; Perchet et al., 2008; Plaghki and Mouraux, 2003). Whenever heat pain thresholds are altered due to central (such as in SCI) rather than peripheral mechanisms, correction for heat pain thresholds is not adequate and was thus not applied in the study of chapter 4. This is because a higher pain threshold in SCI subjects might rather be attributable to altered conduction in central, or possibly peripheral, nerve fibers than to altered heat transfer mechanisms in the skin. Furthermore, the deficits inflicted by the SCI (chapter 4) are so clear, that correction for pain thresholds is not necessary.

6.2 Markers for NP in SCI

The CHEP measurements presented in chapter 4 confirmed the prerequisite of STT dysfunction in the generation of bNP on a more objective basis compared to earlier studies (Defrin et al., 2001; Eide et al., 1996; Finnerup et al., 2003a; Finnerup et al., 2003b; Finnerup et al., 2007). Nevertheless, discrimination between subjects with and without bNP in terms of STT dysfunction was not possible. Although being in accordance with literature, these results did not answer the expectation that CHEP might allow for a more finely scaled assessment of STT function. Clinical examination of the SCI subjects further indicated that subjects with an incomplete SCI suffered more often from bNP. Although this is controversially discussed (Beric et al., 1988; Davidoff et al., 1987; Finnerup et al., 2003b; Siddall et al., 2003; Siddall et al., 1999; Wasner et al., 2008; Werhagen et al., 2004) it could be conceived that the STT damage starts a cascade of changes ultimately leading to bNP in some SCI subjects. In this context, incomplete lesion might be more permissive for axon sprouting.

While CHEP were not different between SCI subjects with and without bNP at a chronic stage, it can not be excluded that differences were present earlier in relation to the time of injury. As presented in chapter 5 the pinprick sensation as measure of STT function improved significantly stronger in subjects with bNP than in those without over the first year after SCI. In accordance with the study in chronic SCI subjects (chapter 4) and with literature no differences were detected at the start and end points. The higher functional improvement in the subjects suffering from bNP was hypothesized to reflect increased spinal plasticity. The presented studies cannot clarify what the exact mechanisms of bNP generation are; nevertheless it is important to note that new therapies which intend to promote motor recovery after SCI could simultaneously induce NP by boosting recovery of STT function. Alarming reports warning of such consequences have been published (Deumens, et al., 2008) and our results strongly support these findings. As the molecular control mechanisms of motor and sensory axonal regeneration are similar, it is difficult to attain one while avoiding the other. Particularly sprouting of primary afferents expressing CGRP should be avoided as it is related to NP behavior (Ackery et al., 2007; Bennett et al., 2000; Christensen and Hulsebosch, 1997). In approaches targeting increased motor recovery, both by diminishing axon growth inhibition or by boosting remyelination, has sprouting of CGRP fibers been detected, for a review see (Deumens et al.,

2008). Furthermore, in one study on intraspinal neural stem cell grafts a close correlation between the strength of the induced allodynia and the extent of CGRP fiber sprouting was found (Hofstetter et al., 2005). Whereas sprouting of sensory neurons may not be desirable, particularly if maladaptive, restoration of descending inhibitory pain control e.g. through raphe nucleus-spinal projections, could be beneficial (Hains et al., 2002; Hains et al., 2001).

The potential read-out parameter for bNP in chronic SCI as determined in chapter 4 is reduction of EEG peak frequency. Although being affected by several mechanisms other than bNP like age or deafferentiation, this parameter is, as presented, going to be sensitive for bNP after normalization for these factors. It is hypothesized that thalamocortical dysrhythmia underlies the reduction in EEG peak frequency. The decreased excitatory input to thalamic nuclei initiates this cascade by hyperpolarization of the cell membrane. The subsequent spike-bursting of thalamic cells could be related to the SCI NP (Lenz et al., 1994). On all accounts the contribution of supraspinal mechanisms to SCI NP might be considerable given the ample changes that the SCI can induce at supraspinal sites (e.g (Abraham et al., 2001; Gerke et al., 2003; Pattany et al., 2002; Zhao et al., 2007b).

6.3 Outlook

In coming interventional studies it will be of utmost importance to supervise bNP development in addition to the functional recovery (Steeves et al., 2007). Additionally, the clinical observation of bNP should be supplemented by measurement of resting EEG to monitor development of EEG peak frequency. Furthermore it should be noted that bNP develops rather late after SCI (1.8±1.7 years, mean±SD (Siddall et al., 2003)) and therefore needs to be supervised for at least three years.

Combination of the data of clinical functional STT recovery (chapter 5) and the CHEP measurements (chapter 4) leads to the need for a longitudinal study observing CHEP course throughout at least the first year after SCI. By conducting a longitudinal study, it could be more thoroughly elucidated whether enhanced recovery of STT function indeed parallels the development of bNP. Such a longitudinal study would need highly homogenous subject groups in terms of SCI lesion at the beginning of the study. The NP type of the included subjects will only be known towards the end of the study. Therefore, a high number of subjects will be required for achieving enough

statistical power – a goal that is only achievable by collaboration with other SCI centers. Additionally, bNP should ideally be followed for three or even five years to reduce the chance that the pain evolves after the observation period.

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8 List of Abbreviations

AIS	American Spinal Cord Injury Association Impairment Scale
bNP	Below level neuropathic pain
CGRP	Calcitonin gene related peptide
CHEP	Contact heat evoked potential
CNS	Central nervous system
CV	Conduction velocity
EEG	Electroencephalogramm
GFP	Global field power
GMD	Global map dissimilarity
NP	Neuropathic pain
SCI	Spinal cord injury
STT	Spinothalamic tract
Thr	Heat pain threshold

Curriculum Vitae

Name	Susanne Wydenkeller
Date of birth	7 July 1981
Citizen of	Winterthur, Switzerland

Education

2006-2009	PhD from the ETH - Swiss Federal Institute of Technology Zurich, Switzerland. Research conducted at the Spinal Cord Injury Center, University Hospital Balgrist, Zurich.
2005-2006	Diploma as Biology Teacher, „Didaktischer Ausweis im Fach Biologie“ ETH - Swiss Federal Institute of Technology Zurich
2006	Cambridge Certificate of Proficiency in English
2001-2005	Master of Science, Specialization in Neurobiology ETH - Swiss Federal Institute of Technology Zurich
1994-2001	Grammar School in Winterthur Kantonsschule Rychenberg, language profile
1988-1994	Primary School in Winterthur

Occupational activities

2007-2009	Part-time job (10%) as Medical Technical Assistant, Neurophysiology Lab, University Hospital Balgrist, Zurich.
2001-2004	Part-time job as administrative staff at the seminar organizer SwissTS Technical Services AG, Wallisellen

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